# CARDIOPROTECTIVE EFFECT OF ETHANOLIC EXTRACT OF BARK OF *Helicteres isora* Linn. ON ISOPROTERENOL INDUCED MYOCARDIAL INFARCTION IN RATS

Dissertation submitted to

**The Tamil Nadu Dr. M.G.R. Medical University, Chennai-32**

In partial fulfillment of the award of the degree of

**MASTER OF PHARMACY IN**

**PHARMACOLOGY**

**Submitted by**

**REG.No.261425220**

**Under the Guidance of**

**Dr. V. RAJESH, M.Pharm,Ph.D.,**

**DEPARTMENT OF PHARMACOLOGY**

**J.K.K. NATTRAJA COLLEGE OF PHARMACY**

**KUMARAPALAYAM– 638 183**

**TAMILNADU**

**OCTOBER-2016**

# EVALUATION CERTIFICATE

This is to certify that the dissertation work entitled **"Cardioprotective Effect of Ethanolic Extract of Bark of *Helicteres isora* Linn. on Isoproterenol Induced Myocardial Infarction in Rats"** submitted by the student bearing **[REG.No.261425220]** to **"The Tamil Nadu Dr. M.G.R.Medical University"**, Chennai, in partial fulfillment for the award of Degree of **Master of Pharmacy** in **Pharmacology** was evaluated by us during the examination held on……

**Internal Examiner** **External Examiner**

# CERTIFICATE

This is to certify that the dissertation **"Cardioprotective Effect of Ethanolic Extract of Bark of *Helicteres isora* Linn. on Isoproterenol Induced Myocardial Infarction in Rats"** is a bonafide work done by **Reg.No.261425220,** Department of Pharmacology, J.K.K. Nattraja College of Pharmacy, Kumarapalayam, in partial fulfillment of the University rules and regulations for award of **Master of Pharmacy** in **Pharmacology** under my guidance and supervision during the academic year 2015-2016.

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This is to certify that the work embodied in this dissertation entitled **"Cardioprotective Effect of Ethanolic Extract of Bark of *Helicteres isora* Linn. on Isoproterenol Induced Myocardial Infarction in Rats",** submitted to "**The TamilNadu Dr.M.G.R. Medical University**", Chennai, in partial fulfillment to the requirement for the award of Degree of **Master of Pharmacy** in Pharmacology, is a bonafide work carried out by **Mr. KARTHIKEYAN K.M.R, [REG.No.261425220]** during the academic year 2015-2016, under the guidance and supervision of **Dr. V. Rajesh, M.Pharm, Ph.D.,** Professor, Department of Pharmacology, J.K.K.Nattraja College of Pharmacy, Kumarapalayam.

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# DECLARATION

I do hereby declared that the dissertation **"Cardioprotective Effect of Ethanolic Extract of Bark of *Helicteres isora* Linn. on Isoproterenol Induced Myocardial Infarction in Rats"** submitted to "**The Tamil Nadu Dr.M.G.R Medical University"**, Chennai, for the partial fulfillment of the degree of **Master of Pharmacy** in **Pharmacology,** It is a bonafide research work has been carried out by me during the academic year 2015-2016, under the guidance and supervision of **Dr. V. Rajesh, M.Pharm, Ph.D.,** Professor, Department of Pharmacology, J.K.K.Nattraja College of Pharmacy, Kumarapalayam.

I further declare that this work is original and this dissertation has not been submitted previously for the award of any other degree, diploma, associate ship and fellowship or any other similar title. The information furnished in this dissertation is genuine to the best of my knowledge.

**Place:** Kumarapalayam **Mr. KARTHIKEYAN K.M.R**

**Date:** **[REG.No.261425220]**

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दाक्षणा क्षत्रीय केन्द्र / Southern Regional Centre
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सं. भा.व.स./द.क्षे.के./ No.BSI/SRC/2/23/2014-2015/1191 दिनांक/Date: 02.12.2015

सेवा में / To

Mr. Karthikeyan. K.M.R
II Year M. Pharm.
Department of Pharmacology
J.K.K. Nattraja College of Pharmacy
Komarapalayam – 638 183
Namakkal Dist., Tamil Nadu

महोदया/Madam,

The plant specimen brought by you for identification is identified as *Helicteres isora* L - MALVACEAE

धन्यवाद/Thanking you,

भवदीय/Yours faithfully,

(डॉ. जी.वी.एस. मूर्ति /Dr.V. Murthy)
वैज्ञानिक 'एफ' एवं कार्यालय अध्यक्ष /
Scientist 'F' & Head of Office

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**Animal Ethical Committee Clearance Certificate**

We, the Undersigned Chairman/Members of the Animal Ethical Committee, functioning in JKK Nattraja College of Pharmacy have studied the proposed research Subject/Project of **KARTHIKEYAN K.M.R.** titled **"Cardioprotective Effect of Ethanolic Extract of Bark of Helicteres isora Linn. on Isoproterenol Induced Myocardial Infarction in Rats"** applying for permission for animal usage and hereby give the certificate of clearance of approval by this Ethical Committee.

Signature of the Chairman/ Members of the Animal Ethical Committee

**Name of the Institution:**

**Station :**

**Date :**

**Seal :**

# ACKNOWLEDGEMENT

I express whole hearted gratitude to my guide **Dr. V. Rajesh, M.Pharm, Ph.D.,** Professor, Department of Pharmacology, J.K.K.Nattraja College of Pharmacy, Kumarapalayam**.**, for suggesting solution to problems faced by me and providing indispensable guidance, tremendous encouragement at each and every step of this dissertation work. Without his critical advice and deep-rooted knowledge, this work would not have been a reality.

I am proud to dedicate my deep sense of gratitude to the founder, (Late) Thiru **J.K.K. Nattaraja Chettiar,** providing us the historical institution to study.

My sincere thanks and respectful regards to our reverent Chairperson **Smt. N. Sendamaraai, B.Com.,** Managing Director **Mr. S. Omm Sharravana, B.Com., LLB.,** J.K.K. Nattraja Educational Institutions, Kumarapalayam for their blessings, encouragement and support at all times.

It is most pleasant duty to thank for our beloved Principal **Dr. R. Sambathkumar, M.Pharm., Ph.D.,** J.K.K.Nattraja College of Pharmacy, Kumarapalayam for ensuring all the facilities were made available to me for the smooth running of this project

Our glorious acknowledgement to our administrative officer **Dr. K.Sengodan, M.B.B.S.**, for encouraging us in a kind and generous manner to complete this work.

My sincere thanks to **Dr. N. Venkateswaramurthy, M.Pharm, Ph.D.,** Professor and Head, Department of Pharmacy Practice. **Dr.Radhika, M.Pharm.,Ph.D.,** Assistant Professor, **Dr. K.Sattanathan, M.Pharm.,Ph.D.,** Assistant Professor, **Ms. Taniya Jacob, M.Pharm.,** Lecturer, Department of Pharmacy Practice, **Ms. V.Viji Queen M.Pharm.,** Lecturer, Department of

Pharmacy Practice **Mrs. K. KrishnaVeni M.Pharm.,** Assistant professor**,** Department of Pharmacy Practice, **Mrs. P. Kavitha M.Pharm.,** Assistant professor, Department of Pharmacy Practice,

My sincere thanks to **Mrs. S. Bhama, M.Pharm.,** Assistant Professor Department of Pharmaceutics, **Mr. R. Kanagasabai, B. Pharm. M.Tech.,** Assistant Professor, **Mr. K. Jaganathan, M.Pharm.,** Lecturer, Department of Pharmaceutics **Mr. C. Kannan M.Pharm.,** Lecturer, Department of Pharmaceutics and **Mr.V. Kamalakannan M.Pharm.,** Lecturer, Department of pharmaceutics for their valuable help during my project.

It is my privilege to express deepest sense of gratitude toward **Dr. M. Vijayabaskaran**, **M.Pharm.,** Professor & Head, Department of Pharmaceutical chemistry and **Mrs. S. Gomathi M.Pharm.,** Lecturer, Department of Pharmaceutical chemistry, **Mrs.B. Vasuki M.Pharm.,** Lecturer, Department of Pharmaceutical chemistry, **Mr.S.P. Vinoth M.Pharm.,Ph.D** Asst. professor, Department of Pharmaceutical chemistry for their valuable suggestions and inspiration.

My sincere thanks to **Dr. V. Sekar, M.Pharm.,Ph.D** Professor and Head, Department of Analysis, **Mr.K.K.Senthil, M.Pharm.,** Lecturer, Department of Analysis and **Mr. S. Jayaseelan, M.Pharm.,** Assistant Professor, Department of Pharmaceutical Analysis for their valuable suggestions.

My sincere thanks to **Dr. Mr. Senthilraja, M.Pharm., Ph.D.,** Professor and Head, Department of Pharmacognosy **Dr. Mr. Rajkumar, M.Pharm., Ph.D..,** Assistant Professor, Department of Pharmacognosy and **Mrs. MeenaPrabha M.Pharm.,** Lecturer, Department of Pharmacognosy for their valuable suggestions during my project work.

My sincere thanks to **Dr. R. Shanmugasundaram, M.Pharm., Ph.D.,** Vice Principal, Professor & Head, Department of Pharmacology, **Mrs. Elavarsi M.Pharm.,** Lecturer, Department of Pharmacology, **Mrs. Babykala M.Pharm.,** Lecturer, Department of Pharmacology, **Mrs. Vannamalar M.Pharm.,** Lecturer,

Department of Pharmacology, **Mrs. Prakash. M.Pharm.,Ph.D.** Asst professor, Department of Pharmacology for their valuable suggestions during my project work.

I greatly acknowledge the help rendered by **Mrs. K. Rani,** Office Superintendent, **Mrs. V. Gandhimathi, M.A., M.L.I.S.,** Librarian, **Mrs S. Jayakala B.A., B.L.I.S.,** Asst. Librarian for their co-operation.

I owe my thanks to all the technical and non-technical staff members of the institute for their precious assistance and help.

Last, but nevertheless, I am thankful to my lovable parents and all my friends for their co-operation, encouragement and help extended to me throughout my project work.

**Mr. KARTHIKEYAN**

**[REG.No.261425220]**

# INDEX

# 1. INTRODUCTION

## HERBAL DRUGS:

Cardiovascular Diseases represent secondary cause of deaths in today's world. Modern drugs although effective in preventing the disorders, are of limited use owing to their adverse reactions. A wide array of plants and its active principles, with minimal side effects, provide an alternate therapy for Ischemic heart disease. Moreover, the plant kingdom represents a largely unexplored reservoir of biologically active compounds.[1]

The herbal medicines are effective in the treatment of various ailments. With only a few exceptions, most herbal treatments have not been tested for safety and efficacy utilizing scientific studies or clinical trials. The herbal drugs are unscientifically exploited and are improperly used. Therefore these plant drugs deserve detailed studies in the light of modern science. The detailed investigation and documentation of plants used in local health traditions and pharmacological evaluation of these plants can lead to the development of invaluable plant drugs for many dreaded diseases.

Herbal medicine is older than any other type of healthcare system with every culture taking advantage of the medicinal values of the herbs and their benefits. Indian System of Medicine including ayurveda, siddha and unani recognized the medicinal uses of plants and plant derived products and herbs before the dawn of modern civilization. Knowledge of medicinal use of plants in India is amassed over a millennium by tribal people. The scientific studies of such plants is carried out all over in India since Vedic times (i.e., more than 6000 years B.C.) beginning with 1800 AD.

Man's knowledge of herbs and their medicinal uses advanced over time and continuous activity was started in isolating active constituents from the medicinal plants in pure state or as crude extracts and screening for its biological activity was started through trial and error methods in addition to observing animals. As a result Herbal pharmacopoeias were developed by different tribes to document all these primitive findings of the activities of the plants. Even the pharmacopoeia of scientific medicine in the 20th century was developed primarily from herbal source.[2]

In the second half of the nineteenth century brought with it a number of important discoveries in the newly developing fields of chemistry and witnessed the rapid progress of this science. Herbal plants became one of the major objects of interest on that time. So many active constituents are isolated from the herbal plants and used for the treatment of many diseases.[3]

World population is nearing 5 billion and with this rate of growth it is likely to touch 7.5 billion in 2020. Global economical survey indicate that over ¾ of the world population in 2020 cannot afford the products of western pharmaceutical industries and have to rely upon the use of traditional medicines which are mainly derived from plants. In accordance with the WHO organization more than 20000 plant species are used for medicinal practices and 80% of the world's total population relies chiefly on herbal traditional medicines to their primary healthcare, even in developed countries. Because herbal plants are comparatively in expensive and produce fewer side effects, herbal plants are great value in the field of treatment and cure of diseases, over the years.[4]

The modern developments in the instrumental analysis and chromatographical methodologies have added numerous complex and rare natural products to the armoury of phytomedicine.

In the western world, as the people are becoming aware of potency and side effects of synthesis drugs, there is an increasing interest in the plant based remedies with a basic approach towards the nature due to les side effects.[5]

Many of the herbal plants are claimed for the cardio protective property and few of the species were scientifically proved by the modern methods they are *Zingiber officinale, Inula racemosa, Ocimum sanctum, Hibiscus rosasinensis, Commiphora mulkul, Punica grantum, Mangifera indica.*

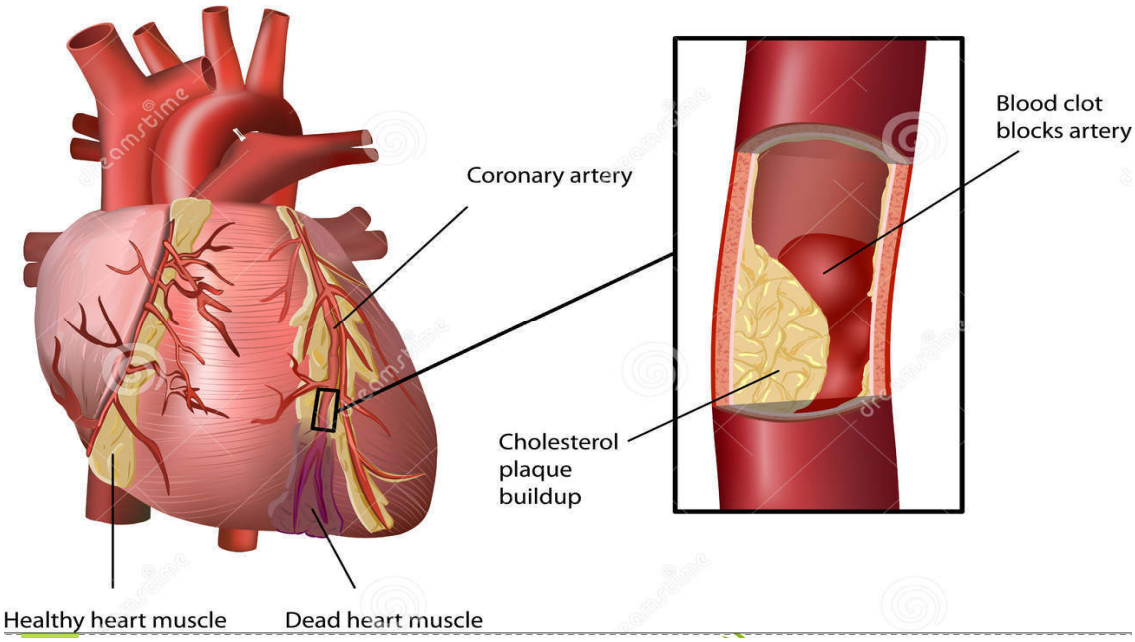
## MYOCARDIAL INFARCTION (MI):

Myocardial infarction (MI) is an acute or chronic form of cardiac disability occurring due to the disparity between the myocardial supply and demand for oxygenated blood.[5] MI causes irreversible necrosis of the heart tissue that is accountable for the principle cause of death in developed and developing countries.[6] Infarcts are most frequently located in left

ventricle than right ventricle. Right ventricle is less susceptible to the infarction due to its thin wall, and less metabolic requirements.

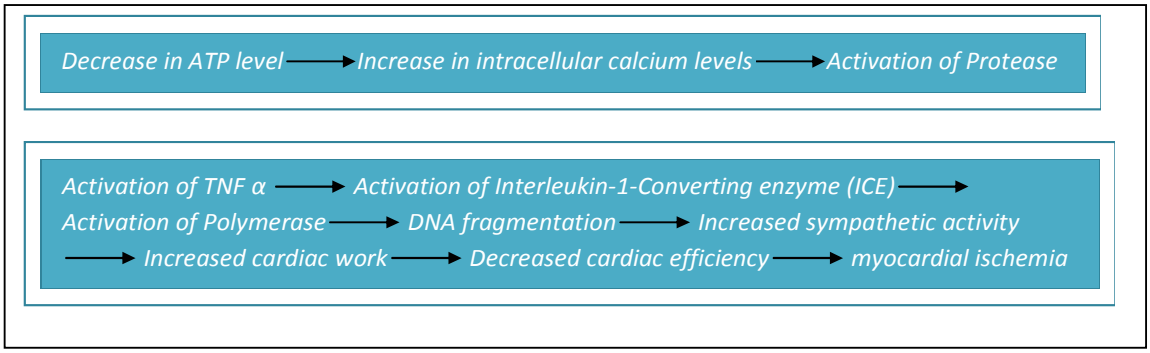
Acute myocardial infarction (MI) remains a leading cause of morbidity and mortality worldwide. Myocardial infarction occurs when myocardial ischemia (diminished blood supply to the heart) occurs, causing interruption in the blood supply to a part of the heart, resulting in the death of heart cells.[7] Critical myocardial ischemia can occur as a result of increased myocardial metabolic demand, decreased delivery of oxygen and nutrients to the myocardium via the coronary circulation, or both. An interruption in the supply of myocardial oxygen and nutrients occurs when a thrombus is superimposed on an ulcerated or unstable atherosclerotic plaque and results in coronary occlusion.[8]

**FIG. 1: Picture Depicting Myocardial Infarction**




Cardiac myocytes mainly rely upon the aerobic metabolism. If the blood supply to the heart decreases; it leads to the death of myocytes. This mechanism is called as Apoptosis or necrosis. This event occurs by two pathways.[7]

**FIG. 2: Mechanism of Apoptosis**




## CLASSIFICATION[9]

Myocardial infarction can be classified on the basis of anatomic, morphologic, and diagnostic clinical information. The two types of MI are

A) Transmural

B) Nontransmural.

**Transmural MI** is characterized by ischemic necrosis of the full thickness of the affected muscle segments, extending from the endocardium through the myocardium to the epicardium.

**Nontransmural MI** is defined as an area of ischemic necrosis that does not extend through the full thickness of myocardial wall segment(s). In a nontransmural MI, the area of ischemic necrosis is limited to the endocardium or to the endocardium and myocardium.

In clinical context, MI is further sub classified into two types: STEMI (ST elevation MI) and non-STEMI (non-ST elevation MI).[10]

In 2007, MI was classified into five major types by a consensus document. They include:[11]

- **Type 1** – Spontaneous MI which occurs due to a primary coronary event such as plaque erosion or rupturing, or fissuring.

- **Type 2** – MI occurring secondary to ischemia which may be due to increased oxygen demand or decreased supply. e.g. coronary embolism, arrhythmias, hypertension.

- **Type 3** – Sudden cardiac death, including cardiac arrest, with symptoms suggestive of ischaemia, taged along by a new ST elevation, or evidence of fresh thrombus in a coronary artery by angiography or autopsy, but death occurring before blood samples could be acquired, or at a time prior to the appearance of cardiac biomarkers in the blood.

- **Type 4** – Associated with coronary angioplasty or stents:

    - **Type 4a** – MI associated with PCI.

    - **Type 4b** – MI associated with stent thrombosis.

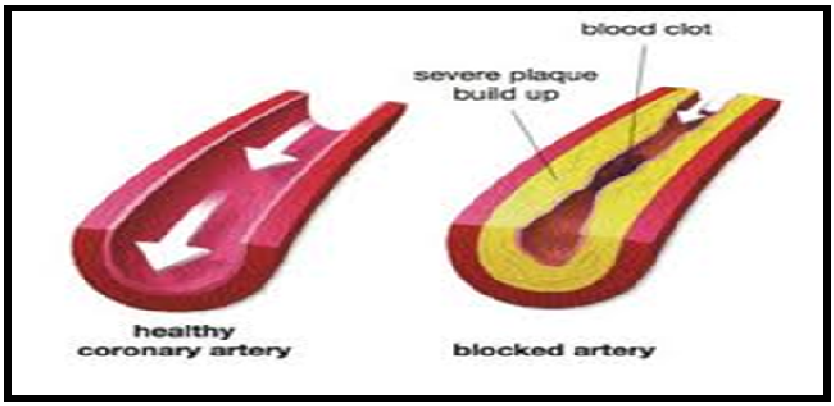
- **Type 5** – MI associated with CABG.

## PATHOPHYSIOLOGY

Pathophysiology of MI involves the formation of atherosclerotic plaques in an epicardial branch of the coronary artery. The development of clot causes decrease in the blood supply to the heart. Sometimes a haemorrhage may occur within the plaque which leads to the occlusion of the vessel. If such an occlusion persists for more than 20 minutes, irreversible myocardial cell damage and cell death will occur.[12]

The atherosclerotic plaque develops over a period of years to decades. The two primary characteristics of the clinically symptomatic atherosclerotic plaque are a fibromuscular cap and an underlying lipid-rich core. Plaque erosion can occur because of the actions of matrix metalloproteases and the release of other collagenases and proteases in the plaque, which result in thinning of the overlying fibromuscular cap.

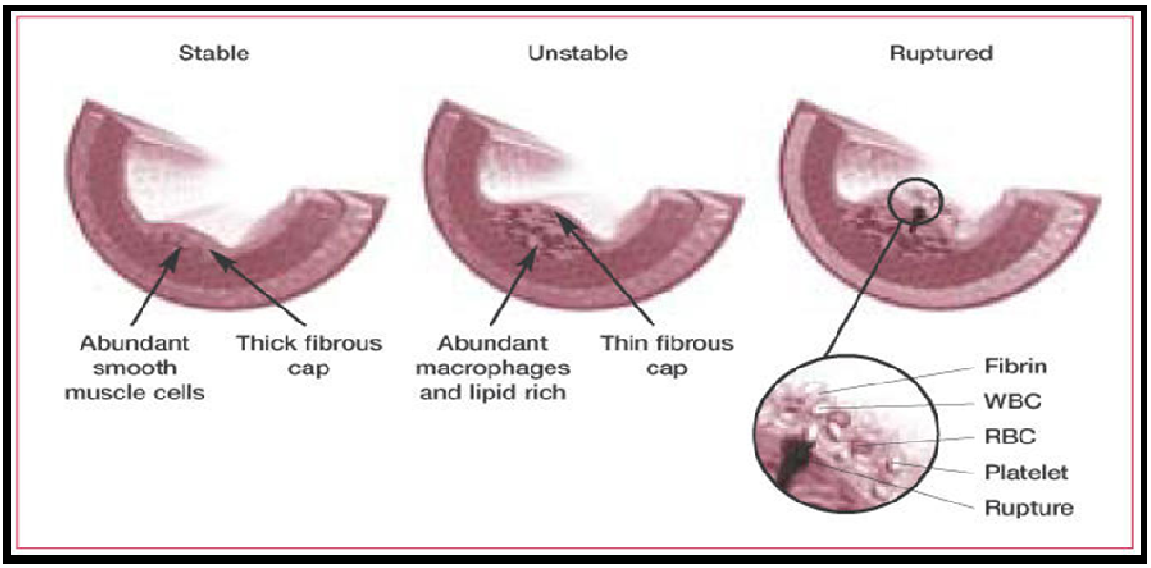
**FIG. 3:Cross Section ofCoronory Artery**




The action of proteases, in addition to hemodynamic forces applied to the arterial segment, can lead to a disruption of the endothelium and fissuring or rupture of the fibromuscular cap. The loss of structural stability of a plaque often occurs at the junction of the fibromuscular cap and the vessel wall, a site otherwise known as the shoulder region. Disruption of the endothelial surface can cause the formation of thrombus via platelet-mediated activation of the coagulation cascade. If a thrombus is large enough to occlude coronary blood flow, an infarct can result.

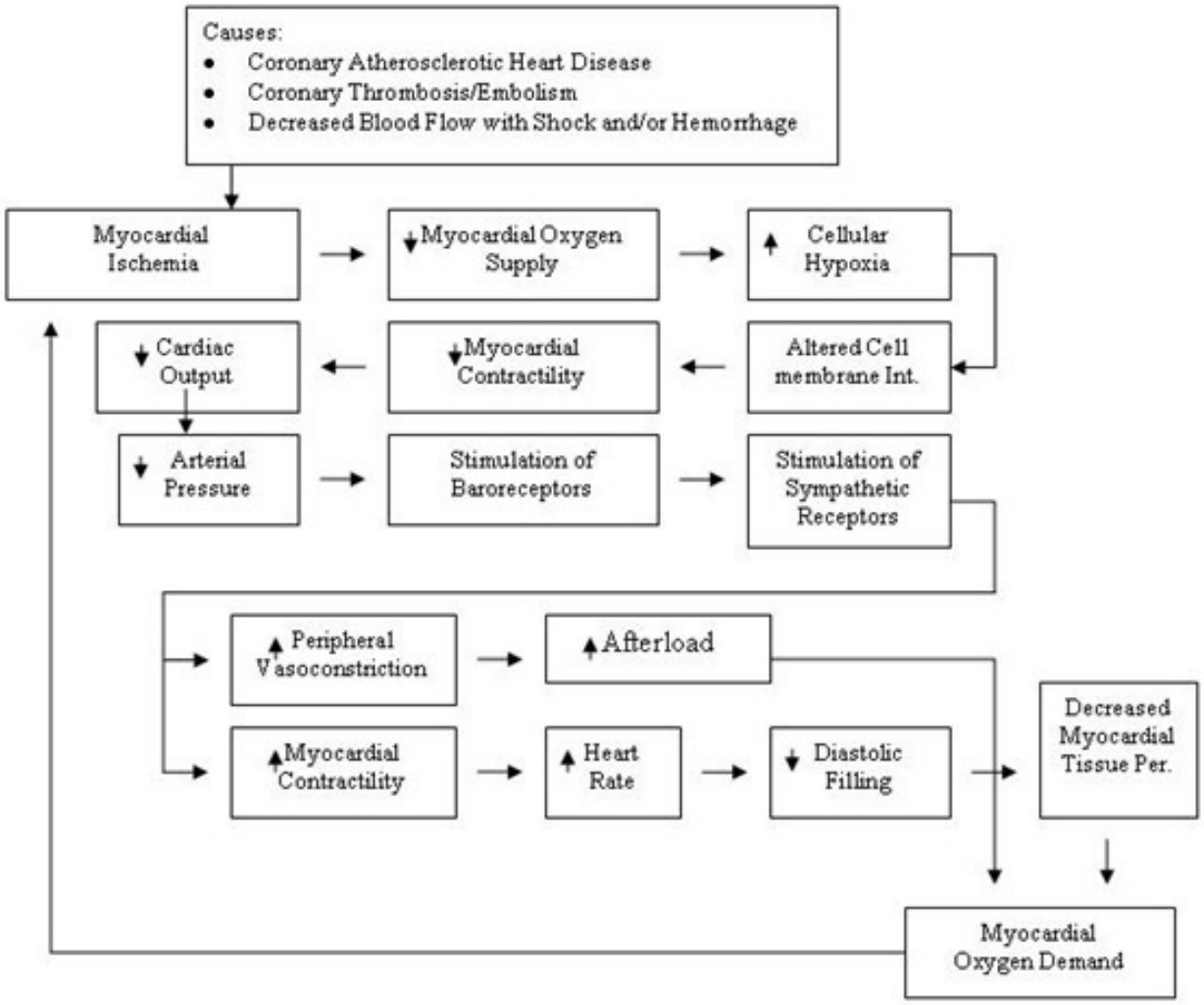
The death of myocardial cells first occurs in the area of myocardium most distal to the arterial blood supply: the endocardium. As the duration of the occlusion increases, the area of myocardial cell death enlarges, extending from the endocardium to the myocardium and ultimately to the epicardium. The area of myocardial cell death then spreads laterally to areas of watershed or collateral perfusion. Generally, after a 6 to 8 hour period of coronary occlusion, most of the distal myocardium has died. The extent of myocardial cell death defines the magnitude of the MI. If blood flow can be restored to at-risk myocardium, more heart muscle can be saved from irreversible damage or death.[13]

**FIG. 4:Development Of Thrombus In Coronory Artery**




The severity of an MI depends on three factors: the level of the occlusion in the coronary artery, the length of time of the occlusion, and the presence or absence of collateral circulation. Generally, the more proximal the coronary occlusion, the extensive amount of myocardium, that will be at risk of necrosis.The larger the myocardial infarction, the greater the chance of death, because of a mechanical complication or pump failure. The longer the period of vessel occlusion, the greater the chances of irreversible myocardial damage distal to the occlusion.[14]

**FIG. 5: Pathophysiology of myocardial infarction**




{Source: nurses blogspot}

## SIGNS AND SYMPTOMS:

The onset of symptoms of MI are gradual, occurring over several minutes and is rarely instantaneous. Most common symptom includes chest pain, a sensation of tightness, pressure or squeezing. Pain radiates to the left arm, lower jaw, neck, back and epigastrium.

Shortness of breath, diaphoresis (excessive sweating), weakness, light-headedness, nausea, vomiting and palpitations are some other symptoms associated with MI. Loss of consciousness and sudden death can occur due to MI.[15]

Atypical symptoms include dyspnoea, fatigue and sleep disturbances. But most of MI cases are silent, without symptoms of pain, which are later on diagnosed using ECG.[16]

Silent MI is seen in the elderly, in patients with DM, patients with a heart transplantation and due to some psychological factors.

## CLINICAL FEATURES OF ISCHEMIA[17]

The term myocardial infarction reflects cell death of cardiac myocytes caused by ischemia, which is the result of a perfusion imbalance between supply and demand. Ischemia in a clinical setting most often can be identified from the patient's history and from the ECG. Possible ischemic symptoms include various combinations of chest, upper extremity, jaw or epigastric discomfort with exertion or at rest. The discomfort associated with acute myocardial infarction usually lasts at least 20 min. often, the discomfort is diffuse, not localized, not positional, not affected by movement of the region, and it may be accompanied by dyspnea, diaphoresis, nausea or syncope.

These symptoms are not specific to myocardial ischemia and can be misdiagnosed and thus attributed to gastrointestinal, neurological, pulmonary or musculoskeletal disorders. Myocardial infarction may occur with atypical symptoms or even without symptoms, being detected only by ECG, biomarker elevations or cardiac imaging.

## ETIOLOGY:

**Excertion:** MI occurs due to intense physical or psychological stress. Severe excertion results in about 5 fold increase in the rate of MI occurrence. People with poor physical stamina are 30-fold more susceptible when compared to their healthy counterparts. The mechanism of MI due to excertion is an increase in pulse pressure, which results in stretching of arterial walls. Stretching results in increased stress on artheroma, causing breakage and thereby release of debris which floats and eventually clogs major coronary arteries.

**Underlying disease:** Presence of underlying diseases like pneumonia, results in MI. But evidence is not supportive enough to draw conclusions for *Chlamydophila pneumonia* to be considered as a causative factor.[18]

**Age:** Increase in age is considered as a cause for MI. Elderly people are more prone to MI.

## DIAGNOSIS:

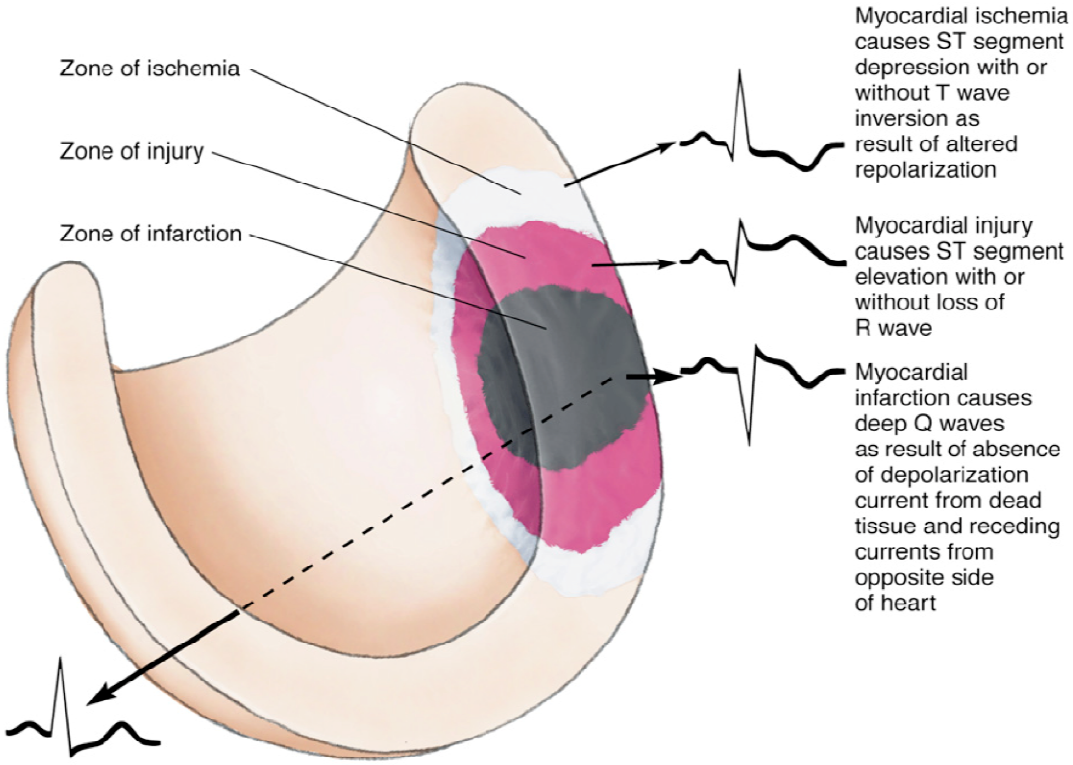
The diagnosis of MI is performed after assessing patient's complaints and performing a general physical examination. Changes in the ECG, coronary angiogram and the levels of cardiac biomarkers help to confirm the diagnosis of MI. Through ECG, the site of myocardial damage can be identified.

Firstly, a chest radiograph and routine blood test will be done to diagnose MI. An echocardiogram also confirms the presence of MI. Imaging techniques such as myocardial perfusion imaging, stress echocardiography are used to confirm MI.[19]

Patients who are stable can be diagnosed using Technetium (99mTc) sestamibi ("MIBI scan") or thallium-201 chloride can be used in nuclear medicine to visualize areas of reduced blood flow in combination with physiologic or pharmacologic stress.

### Echocardiography[20]

Echocardiography is an outstanding real-time imaging technique with reasonable spatial and temporal resolution. Its strong point is the evaluation of myocardial thickness, thickening and motion at rest. This can be aided by tissue Doppler imaging. Echocardiographic contrast agents can advance endocardial visualization, but contrast studies are not yetfully authenticated for the detection of myocardial necrosis, although early work is heartening.




## Radionuclide imaging[21]

Several radionuclide tracers permit viable myocytes to be imaged unswervingly, including thallium-201, technetium-99m MIBI, tetrofosmin, and [18F] 2-fluorodeoxyglucose (FDG). The vigor of the techniques are that they are the only regularly available direct methods of evaluating viability, although the moderately low resolution of the images disadvantages them for perceiving small areas of infarction. The regular single photon-emitting radio-pharmaceuticals are also tracers of myocardial perfusion and so the techniques readily sense areas of infarction and inducible perfusion abnormalities. ECG-gated imaging offers are liable assessment of myocardial motion, thickening and global function.

## Magnetic resonance imaging[22]

Cardiovascular MRI has elevated spatial resolution and sensible temporal resolution. It is a well-validated standard for the evaluation of myocardial function and has, in speculation, similar potential to echocardiography in alleged acute infarction. It is, nevertheless, more unwieldy in an acute setting and is not frequently used. Paramagnetic contrast agents can be used to evaluate myocardial perfusion and the increase in extracellular

space linked with the fibrosis of chronic infarction. The former is not yet fully validated in clinical practice, but the latter is well validated and can play an imperative role in the detection of infarction.

## X-Ray computed tomography[23]

Infarcted myocardium is originally visible to CT as a focal area of diminished LV enhancement, but later imaging shows hyper improvement as with late gadolinium imaging by MRI. This verdict is clinically pertinent because contrast-enhanced CT may be executed for alleged embolism and aortic dissection, circumstances with clinical features that overlap with those of acute myocardial infarction.

## CRITERIA FOR MYOCARDIAL INFARCTION[17]

### CRITERIA FOR ACUTE MYOCARDIAL INFARCTION:

The term myocardial infarction should be used when there is evidence of myocardial necrosis in a clinical setting consistent with myocardial ischemia. Under these conditions any one of the following criteria meets the diagnosis of MI.

- Detection of risk and/or fall of cardiac biomarkers (preferably troponin) with at least one value above the 99$^{th}$ percentile of the upper reference limit together with evidence of myocardial ischemia with atleast one of the following:
    - Symptoms of ischemia;
    - ECG changes indicative of new ischemia [new ST-T changes or new left bundle branch block(LBBB)];
    - Development of pathological Q waves in ECG;
    - Imaging evidence of new loss of viable myocardium or new regional wall motion abnormality;
- Sudden, unexpected cardiac death, involving cardiac arrest, often with symptoms suggestive of myocardial ischemia, accompanied by presumably new ST elevation, or new LBBB, and/or evidence of fresh thrombus by cardiac

angiography and/or at autopsy, but death occurring before blood samples could be obtained, or at a time before the appearance of cardiac biomarkers in blood.

- For percutaneous coronary interventions (PCI) in patients with normal baseline trroponin values, elevations of cardiac biomarkers above the 99$^{th}$ percentile URL are indicative of peri-procedural myocardial necrosis. By convention, increase of biomarkers greater than 3 x 99$^{th}$ percentile URL have been designated as defining PCI related myocardial infarction. A subtype related to a documented stent thrombosis is recognized.

- For coronary artery bypass grafting (CABG) in patients with normal baseline troponin values, elevations of cardiac biomarkers above 99$^{th}$ percentile URL are indicative of peri-procedural myocardial necrosis. By convention increase in biomarkers greater than 5 x 99$^{th}$ percentile URL plus either new pathological Q waves or new LBBB, or angiographically documented new graft or native coronary artery occlusion, or imaging evidence of new loss of viable myocardium have been designated as defining CABG related myocardial infarction.

- Pathological findings of an acute MI.

**CRITERIA FOR PRIOR MYOCARDIAL INFARCTION**

Any one of the following criteria meets the diagnosis of prior MI:

- Development of new pathological Q waves with or without symptoms.

- Imaging evidence of a region of loss of viable myocardium that is thinned and fails to contract, in the absence of non-ischemic cause.

- Pathological findings of a healed or healing myocardial infarction.

## ELECTROCARDIOGRAPHIC DETECTION OF MYOCARDIAL INFARCTION[24, 25, 26, 27]

The ECG is an integral part of the diagnostic work-up of patients with suspected MI and should be acquired and interpreted promptly after clinical presentation. Dynamic changes in the ECG waveforms during acute myocardial ischemic episodes often require acquisition

of multiple ECGs, particularly if the ECG at initial presentation is non-diagnostic. Serial recordings in symptomatic patients with an initial non-diagnostic ECG should be performed at 15–30 min intervals or, if available, continuous computer-assisted 12-lead ECG recording.

Recurrence of symptoms after an asymptomatic interval are an indication for a repeat tracing and, in patients with evolving ECG abnormalities, a pre-discharge ECG should be acquired as a baseline for future comparison. Acute or evolving changes in the ST-T waveforms and Q waves, when present, potentially allow the clinician to time the event, to identify the infarct-related artery, to estimate the amount of myocardium at risk as well as prognosis and to determine therapeutic strategy. More profound ST-segment shift or T wave inversion involving multiple leads/territories is associated with a greater degree of myocardial ischemia and a worse prognosis.

Other ECG signs associated with acute myocardial ischemia include cardiac arrhythmias, intraventricular and atrioventricular conduction delays, and loss of pre-cordial R wave amplitude. Coronary artery size and distribution of arterial segments, collateral vessels, location, extent and severity of coronary stenosis, and prior myocardial necrosis can all impact ECG manifestations of myocardial ischemia. Therefore the ECG at presentation should always be compared to prior ECG tracings, when available. The ECG by itself is often insufficient to diagnose acute myocardial ischemia or infarction, since ST deviation may be observed in other conditions, such as acute pericarditis, left ventricular hypertrophy (LVH), left bundle branch block (LBBB), Brugada syndrome, stress cardiomyopathy, and early repolarization patterns.

Prolonged new ST segment elevation, particularly when associated with reciprocal ST-segment depression, usually reflects acute coronary occlusion and results in myocardial injury with necrosis. As in cardiomyopathy, Q waves may also occur due to myocardial fibrosis in the absence of CAD. ECG abnormalities of myocardial ischemia or infarction may be inscribed in the PR segment, the QRS complex, the ST-segment or the T wave. The earliest manifestations of myocardial ischemia are typically T wave and ST-segment changes. Increased hyperacute T wave amplitude, with prominent symmetrical T waves in at least two contiguous leads, is an early sign that may precede the elevation of the ST-segment. Transient Q waves may be observed during an episode of acute ischemia or (rarely) during acute MI with successful reperfusion.

## COMPLICATIONS

Complications with MI may occur immediately or may need time to develop.

Acute complications include:

- Heart failure
- Aneurysm
- Mitral regurgitation
- Arrhythmias

Long term complications include:

- Heart failure
- Atrial fibrillation
- Risk of second MI

## RISK FACTORS

The incidence of MI is dependent on predisposing risk factors for atherosclerosis. Smoking, lack of exercise and stress appear to be the major risk factors in the occurrence of MI.[28] Other primary risk factors found to be related to development of atherosclerotic coronary artery disease and MI include:[29, 30]

- Age
- Gender: At any given age, men are more susceptible to MI than women.
- Hyperlipidemia, dyslipidemia, hypercholesterolemia, obesity
- Diabetes mellitus (type 1 and type 2)
- Hypertension , psychological stress
- Tobacco and alcohol
- Familial history of atherosclerotic arterial disease.
- Exposure to pollutants like CO, $NO_2$
- Lack of physical activity

- Long term use of Oral Contraceptive Pill

The presence of any risk factor is associated with doubling the relative risk of developing atherosclerotic coronary artery disease. Many of these risk factors are modifiable, so that MI can be prevented by maintaining a healthy lifestyle.

## TREATMENT

### Vasodilatation

In the treatment of MI organic nitrates like Nitroglycerine, Isosorbidedinitrate are used to decrease the ultimate size infarct and preserves the viable tissues by reducing the oxygen demand of the myocardium.[7]

### Sympathetic activity

In this condition due to the increase in the sympathetic activity oxygen consumption will be increased. β-blockers will reduces the sympathetic activity and decreases the oxygen consumption.[13]

### ACE inhibitors

ACE inhibitors increases the increases the cardiac work. These agents reduce the long-time mortality.[13]

### Calcium channel blockers

Calcium channel blockers are used to reduce the intracellular calcium level. $Ca^{2+}$ agents will alters the contraction of cardiac and smooth muscle contraction by blocking the entry of the $ca^{2+}$ into the myocytes.[7]

### Analgesics

In this condition opioid analgesic like morphine injection is used to decrease the pain and apprehension.[13]

**Thrombolysis**

Thrombolytic agents has been shown to reduce mortality from MI Aspirin irreversibly interferes with function of cyclooxygenase and inhibits the formation of thromboxane A2. Within minutes, aspirin prevents additional platelet activation and interferes with platelet adhesion and cohesion. This effect benefits all patients with acute coronary syndromes, and Myocardial infarction.[7]

**Newer methods of treatment**

Enzymes present all over the body changes which occurs in these enzymes levels will helps in the identifying the disease. In the myocardial infarction the markers enzymes like CK-MB, LDH, ALT, AST is increased. By measuring these enzyme levels the severity of the disease is measured.

Current treatment for acute myocardial infarction (AMI) is aimed at limiting the duration of the ischemic period by disrupting the occlusion in the coronary artery. δ PKC inhibition reduces reperfusion injury to the myocardium at least in part by inhibiting apoptosis. δ PKC inhibition greatly reduced reperfusion-induced cell necrosis, as evidenced by a 5-fold decline in troponin T release. Oxidative stress seems to cause δ PKC translocation to the mitochondria. Therefore, activated δ PKC at the mitochondria may have direct effects on protein substrates involved in mitochondrial energetics and pHregulation as well as in apoptosis.

Patients receiving stem cell treatment as coronary artery injections of stem cells derived from their own bone marrow after a MI show improvement in left ventricular ejection fraction and end-diastolic fraction. The larger the initial infarct size, the greater the effect of the infusion. Clinical trials in this direction are initiating.[31]

Currently there are three biomaterial and tissue engineering approaches for the management of MI, but these are in very earlier stages of medical research. They include; polymeric left ventricular restraints in the prevention of heart failure, *in vitro* engineered cardiac tissue, which is subsequently, implanted *in vivo* and injecting cells or a scaffold into the myocardium to create *in situ* engineered cardiac tissue.[32]

## PROGNOSIS

An individual patient's long-term outcome following an MI depends on numerous variables, some of which are not modifiable from a clinical standpoint. However, patients can modify other variables by complying with prescribed therapy and adopting lifestyle changes.

### *Stress testing*

Stress testing is not recommended within several days after a myocardial infarction. Only submaximal stress tests should be performed in stable patients 4 to 7 days after MI. Symptom-limited stress tests are recommended 14 to 21 days after an MI.[33]

## LONG-TERM MEDICATIONS

Most oral medications instituted in the hospital at the time of MI will be continued long term. Therapy with aspirin and beta blockade is continued indefinitely in all patients. ACE inhibitors are continued indefinitely in patients with congestive Heart failure, Left ventricular Dysfunction, Hypertension, or Diabetes. A lipid-lowering agent, specifically a statin, in addition to diet modification, is continued indefinitely as well. Post-MI patients with diabetes should have tight glycemic control according to earlier studies.[9]

### Cardiac rehabilitation

Cardiac rehabilitation provides a venue for continued education, reinforcement of lifestyle modification, and adherence to a comprehensive prescription of therapies for recovery from MI including exercise training. Participation in cardiac rehabilitation programs after MI is associated with decreases in subsequent cardiac morbidity and mortality. Other benefits include improvements in quality of life, functional capacity, and social support. However, only a minority of post-MI patients actually participate in formal cardiac rehabilitation programs because of several factors, including lack of physician referrals, low patient motivation, noncompliance, and financial constraints.[34]

# EPIDEMIOLOGY

Myocardial infarction is a common presentation of heart ischemic heart disease. The WHO estimated that in the year of 2002, 12.6% of the death all over the world is by ischemic heart disease. And it is the leading cause of death in developed countries.

In the United States, heart diseases are the major cause of death and they are having higher mortality rate than cancer. Heart disease is responsible for 1 in 5 death in U.S. Around 72,00000 men and 60,00000 women in U.S affected by the heart disease.

In India Cardiovascular diseases is the leading for the death in the year of 2007. In the year of 1990 the death is around 1.17 million and it is raised to 1.59 million in 2010. It became quickly a major health issue with deaths due to heart diseases during 1985-2015.The mortality rate in India due to heart disease will differs state to state in Meghalaya 49%, Punjab 49%, Goa 42%, Tamilnadu 36%, Andhra Pradesh 31%.

## ANTIOXIDANTS AND MYOCARDIAL INFARCTION

## REDOX IMBALANCE AND MYOCARDIAL ISCHEMIA REPERFUSION INJURY[35, 36, 37, 38, 39]

Inadequate blood supply to a region of the body for a certain period followed by the resumption of blood flow is termed ischemia-reperfusion. Ischemia-reperfusion results in varying degrees of tissue damage depending on the duration and extent of the hypoperfusion. Myocardial damage induced by ischemia-reperfusion is due, at least in part, to the generation of ROS.

Evidence supporting ROS as a culprit of myocardial IR injury came from several direct and indirect observations. There have been reports showing a close correlation between the production of ROS and simultaneous consumption of endogenous antioxidants. Indirect evidence consistent withthis view is the cardioprotective effects of free radical scavengers and antioxidant supplements. In addition, direct genetic manipulations to over expressor under express genes participating in the antioxidant defense also exhibit profound influence on the outcome of IR injury.

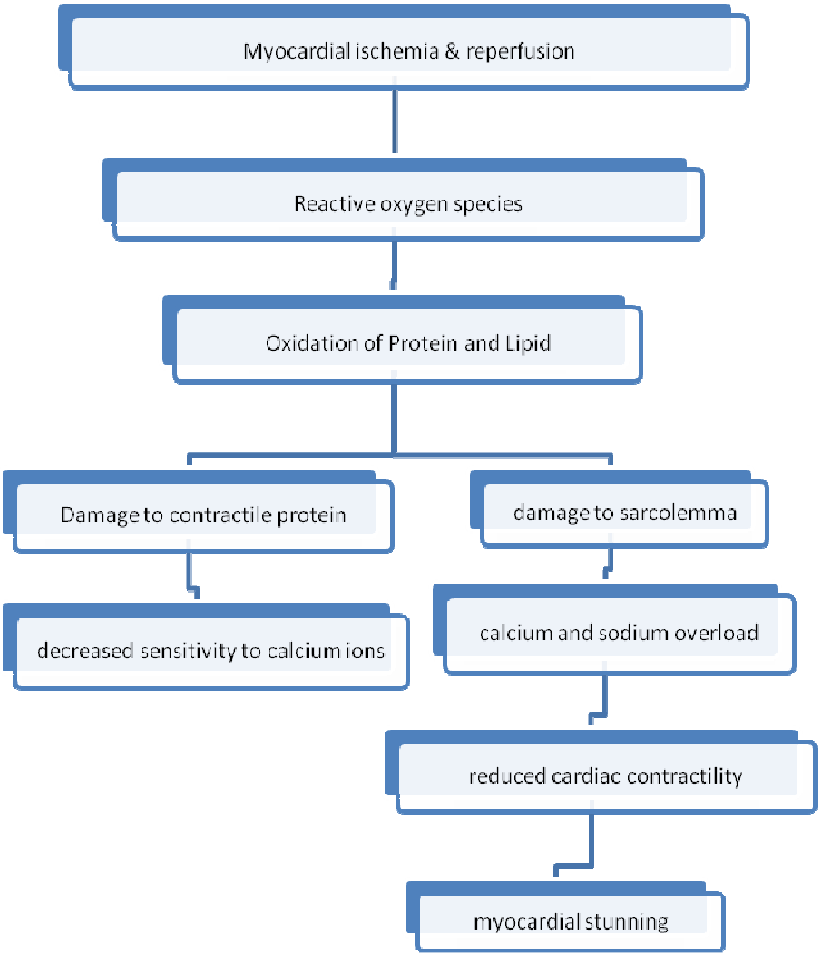
It would seem that the link between ROS and IR injury has appeared to have been clear-cut; however, contradicting results have been reported regarding the effects of antioxidants on IR injury. Inadequate perfusion of a tissue/organ leads to oxygen ($O_2$) and adenosine triphosphate (ATP) depletion, and the accumulation of toxic metabolites.Another effect of hypoperfusion is the conversion of xanthine dehydrogenase to xanthine oxidase, which upon reperfusion, catalyzes the conversion of hypoxanthine to xanthine with the concomitant production of ROS.

Oxygen radicals ($O^{\bullet 2-}$) are also produced by the electron transport system of the mitochondria and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase. The highly toxic ROS are converted to hydrogen peroxide ($H_2O_2$) by superoxide dismutase (SOD), and then to $H_2O$ by catalase and/or glutathione oxidase. However, under ischemic conditions, the endogenous antioxidant system is eroded and the tendency for metal ion assisted conversion of $H_2O_2$ into the destructive hydroxyl radical ($OH^{\bullet -}$) is increased. Accumulated evidence has shown that ROS production is a key event in reperfusion injury when oxygen is reintroduced to ischemic tissues. ROS, especially hydroxyl radical, cause the oxidation of proteins, lipids and nucleic acids, resulting in the structural and functional changes of proteins, disruption of membrane integrity, and genetic mutations, respectively.

## ISCHEMIA REPERFUSION INJURY TO VASCULAR ENDOTHELIUM

ROS increase in concentration upon reperfusion of the ischemic myocardium.[40] The formation of ROS exerts oxidative stress to the myocardium that may cause heart failure. The major ROS that are responsible for the oxidative stress are superoxide anion ($O^{\bullet 2-}$), hydroxyl radical ($OH^{\bullet -}$) and $H_2O_2$ In the vascular walls, the enzyme systems involved in the production of these radicals including xanthine oxidase, NADPH oxidase and the endothelial nitric oxide (NO) synthase (eNOS). Because of its location, the endothelium is probably the prime target for ROS damage.

**FIG. 6: Possible mechanism underlying ischemia reperfusion induced myocardial contractile stunning**[41]



# 2. REVIEW OF LITERATURE

- **Abbas AM (2016)**[42] analyzed the effects of pretreatment with isorhapontigenin and omega-3 FA on rat model of isoproterenol-induced MI. Fifty-six rats were divided into seven groups. Serum levels of cardiac marker enzymes and lipid profile as well as cardiac levels of malondialdehyde and anti-oxidants were measured in all rats. ECG and histopathological examination were performed. Isoproterenol caused a significant elevation of ST segment, decreased R wave amplitude, HDL, and anti-oxidants, and increased LDH, CK-MB, cTnI, TNF-α, interleukin-6, malondialdehyde, triglycerides, T.Ch, LDL, and phospholipids. Omega-3 FA or isorhapontigenin significantly decreased the ST segment elevation, LDH, CK-MB, cTnI, TNF-α, interleukin-6, malondialdehyde, and phospholipids and increased R wave amplitude and anti-oxidants. The effects of combined omega-3 FA and isorhapontigenin were more significant than either of them alone. Therefore, we conclude that omega-3 FA and isorhapontigenin have a cardioprotective effect on rats with isoproterenol-induced MI through their anti-oxidant and anti-inflammatory actions.

- **Hassan MQ et al., (2016)**[43] had enquired whether combination therapy of low-dose benidipine with the potent free radical scavenger edaravone has a cardioprotective effect against isoproterenol (ISO)-induced myocardial infarction (MI) in Wistar rats. Rats were pretreated with concurrent doses of benidipine and edaravone by i.v. and i.p. routes respectively for 28 days, followed by MI induction using ISO (85 mg/kg) by subcutaneous route for two days at 24 h intervals. After the treatment period, blood was withdrawn and the heart was preserved for biochemical estimations. The activities of the cardiac biomarkers (lactate dehydrogenase and CK-MB), and the level of malondialdehyde (MDA) significantly increased, while antioxidant markers were significantly decreased in the ISO intoxicated group compared with the control group. Moreover, the level of C-reactive protein (CRP) and Caspase-3 activity significantly increased in ISO-intoxicated group. An ultrastructure study was also carried out. Pretreatment with a combination of benidipine and edaravone significantly attenuated the activities of the cardiac biomarkers and the level of MDA, and significantly increased the antioxidant markers compared with the ISO-intoxicated group. Furthermore, pretreatment with the combination of benidipine and edaravone significantly decreased the level of CRP and Caspase-3 activity as

compared to the ISO-treated group. The ultrastructure study of myocardium revealed that pretreated groups preserved the mitochondrial shape, the membrane and its internal structures. Taken together these results suggest that the combination of benidipine and edaravone showed significant protective effect in ISO-induced MI.

- **Kumar M et al., (2016)**[44] scrutinized the cardioprotective effects of baicalein, main bioactive constituent from roots of *Scutellaria baicalensis* and *Scutellaria lateriflora*, on isoproterenol (ISO) induced acute myocardial infarction model in rats and to explore the underlying mechanisms. Rats were treated with baicalein (50 mg/kg and 100 mg/kg) orally for 14 days and on 13th and 14th day, myocardial injury was induced by ISO injection (100 mg/kg, subcutaneous) at an interval of 24 h. The study showed that ISO administration resulted in significant elevations in the levels of cardiac injury biomarkers such as cardiac troponin I, CK-MB, AST and ALT. Concentrations of reactive nitrogen species and reactive oxygen species in the heart tissue increased significantly while antioxidant enzymes level declined. The levels of tissue pro-inflammatory cytokines tumor necrosis factor-α and interleukin-6 were significantly increased after ISO administration. Pretreatment with baicalein significantly reversed these alterations induced by ISO administration. Exploration of the underlying mechanisms of protective effect of baicalein pretreatment revealed that it repressed the expression of nuclear factor kappa B and restored the ISO induced elevation of pro-inflammatory cytokines, oxidative and nitrosative stress. It was found that baicalein pretreatment enhanced the level of antioxidant defense enzymes like SOD, catalase and GSH. These findings demonstrated that baicalein pretreatment might have a potential benefit in prevention and terminating ischemic heart diseases like myocardial infarction.

- **Panda V et al., (2016)**[45] had explored the cardioprotective activity of the Macrotyloma uniflorum seed extract (MUSE) and its phenolic acids (p-coumaric acid and ferulic acid) in isoproterenol (ISO)-induced myocardial infarction in rats. The previously mentioned phenolic acids were isolated and quantified from MUSE by HPLC. Pretreatment of gemfibrozil (reference standard), MUSE (250 and 500 mg/kg) and the phenolic acids for 30 days to rats treated with ISO (85mg/kg) on the last 2days resulted in a significant attenuation of the ISO-elevated levels of serum marker enzymes (aspartate aminotransferase, lactate dehydrogenase and creatine

phosphokinase MB), total cholesterol, triglycerides, uric acid, C-reactive protein and malondialdehyde and a restoration of the levels of the ISO-depleted marker enzymes, reduced glutathione and the antioxidant enzymes-superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase in heart. Restoration of the ISO-altered electrocardiogram pattern and haemodynamic parameters (left ventricular end diastolic pressure, heart rate, systolic, diastolic and mean arterial pressure) was also brought about by treatment with MUSE and the phenolic acids. It may be concluded that MUSE treatment to ISO-challenged rats exhibits a significant cardioprotective effect probably because of the potent antioxidant activity of its phenolic acids that salvage the myocardium from the deleterious effects of ISO.

- **Derbali A et al., (2015)**[46] had designed the study to evaluate the cardioprotective effect of Tunisian flaxseed oil (Linum usitatissimum) against isoproterenol-induced myocardial infarction in rats by studying hypertensive and cardiac damage markers especially electrocardiographic changes and troponin T serum level. In vitro, the extracted oil showed an important inhibition of angiotensin converting enzyme (ACE) with an IC50=85.96 μg/ml. According to chemical analysis, this extract is composed essentially of alpha linolenic acid (ALA), an n-3 polyunsaturated fatty acid (58.59 %). Male rats were randomly divided into three groups, namely control (C), isoproterenol (ISO), and isoproterenol-treated group with flaxseed oil (FO + ISO). Isoproterenol injection showed changes in ECG pattern, including ST-segment elevation (diagnostic of myocardial infarction), increase in the serum levels of Troponin T and cardiac injury markers (creatine kinase-MB (CK-MB), lactate dehydrogenase (LDH), alkaline phosphatase (ALP), aspartate transaminase (AST), and alanine transaminase (ALT)). However, Linum oil pre-co-treatment prevented almost all the parameters isoproterenol-induced myocardial infarction in rats. Results of the present study proved that flaxseed oil has a significant effect by heart protection against isoproterenol-induced myocardial infarction through beneficial effect of the important fraction of ALA.

- **Geng ZH et al., (2015)**[47] had conducted a study to investigate the cardioprotective effect of one purified polysaccharide (SMP1) from Salvia miltiorrhiza on isoproterenol (ISO)-induced myocardial infarction (MI) in rats. ISO-treated rats showed severe myocardial damage and high lipid peroxidation level, as well as

decreased endogenous myocardial antioxidant function. Pretreatment with SMP1 (100 and 400mg/kg) for 30 days significantly increased the body weight, decreased the heart weight, attenuated the serum levels of creatine kinase (CK), creatine phospokinase-MB (CK-MB), dehydrogenase (LDH), alkaline phosphate (ALP), aspartate transaminase (AST), alanine transaminase (ALT), total cholesterol, triglyceride, and LDL-cholesterol (LDL-C), along with the increased concentration of HDL-cholesterol (HDL-C). In addition, SMP1 also enhanced myocardial superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX) activities and elevated myocardial reduced glutathione (GSH) level, along with a decrease in thiobarbituric acid reactive substances (TBARS) concentration. Collectively, our results indicated that long-term oral administration of SMP1 offered significant protection against the damage induced by ISO in rat heart through enhancement of endogenous antioxidants and antihyperlipidemic activity.

- **Goyal SN et al., (2015)**[48] had researched the effect of cardamom on hemodynamic, biochemical, histopathological and ultrastructural changes in isoproterenol (ISO)-induced myocardial infarction. Wistar male albino rats were randomly divided and treated with extract of cardamom (100 and 200 mg/kg per oral) or normal saline for 30 days with concomitant administration of ISO (85 mg/kg, subcutaneous) on 29th and 30th days, at 24 h interval. ISO injections to rats caused cardiac dysfunction evidenced by declined arterial pressure indices, heart rate, contractility and relaxation along with increased preload. ISO also caused a significant decrease in endogenous antioxidants, superoxide dismutase, catalase, glutathione peroxidase, depletion of cardiomyocytes enzymes, creatine kinase-MB, lactate dehydrogenase and increase in lipid peroxidation. All these changes in cardiac and left ventricular function as well as endogenous antioxidants, lipid peroxidation and myocyte enzymes were ameliorated when the rats were pretreated with cardamom. Additionally, the protective effects were strengthened by improved histopathology and ultrastructural changes, which specifies the salvage of cardiomyocytes from the deleterious effects of ISO. The present study findings demonstrate that cardamom significantly protects the myocardium and exerts cardioprotective effects by free radical scavenging and antioxidant activities.

- **Kavitha S et al., (2015)**[49] had inspected and found amelioration of methanolic extract of *O. sanctum* (Tulsi) leaves on inflammation in isoproterenol (ISP) induced MI in rats. Myocardial infarction (MI) is one of the leading causes of death worldwide. Oxidative stress and inflammation play vital role in the development of MI. The Indian basil or Tulsi (*Ocimum sanctum Linn*.), owing to its antioxidant potential, is used in the traditional system of Indian medicine to treat various disorders. ISP-induced MI increased the levels of cardiac markers, phospholipases and phospholipid content. However, the same were reduced on pre-treatment with methanolic extract of O. sanctum leaves. The activities of 5-lipoxygenase and cycloxygenase-2 and levels of leukotriene B4 and thromboxane B2 were also elevated in ISP-treated rats, which were significantly decreased ($P < 0.001$) in extract pre-treated rats. The enhanced mRNA expressions of nuclear factor kappa-B, 5-lipoxygenase activating protein and receptor for leukotriene B4 on MI induction, were considerably reduced ($P < 0.001$) on extract pre-treatment. Histopathological analysis also confirmed the findings. The results also revealed the high phenolic content of methanolic extract of *O. sanctum* leaves. The study demonstrated that methanolic extract of Tulsi leaves can decrease inflammation in the cardiac tissue of ISP-induced MI in rats and its effect may be through downregulation of oxidative stress and arachidonic acid pathway. This cardioprotective effect may be due to the high phenolic content of methanolic extract of O. sanctum leaves.

- **Khalil MI et al., (2015)**[50] had explored the protective role of *Withaniasomnifera* leaf extract (WSLEt) on isoproterenol- (ISO-) induced myocardial infarction (MI) in rats. Subcutaneous injection of ISO (85 mg/kg body weight (b.w.)) administered to rats for two consecutive days caused a significant increase in cardiac troponin I (cTnI) levels and serum lipid profiles, as well as the activities of some marker enzymes. In addition to these diagnostic markers, there were increased levels of lipid peroxidation (LPO) and decreased activities of enzymatic antioxidants (superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GRx), and glutathione-S-transferase (GST)) in the myocardium. However, oral pretreatment (100 mg/kg b.w.) with WSLEt for 4 weeks elicited a significant cardioprotective activity by lowering the levels of cTnI, lipid profiles, and marker enzymes. The levels of LPO products were also significantly decreased. Elevated activities of antioxidant enzymes were also observed in rats pretreated with WSLEt. As further confirmed

histopathologically, our findings strongly suggest that the cardioprotective effect of WSLEt on myocardium experiencing ISO-induced oxidative damage may be due to an augmentation of the endogenous antioxidant system and an inhibition of LPO in the myocardial membrane. We conclude that WSLEt confers some protection against oxidative damage in ISO-induced MI in rats.

- **Sun SJ et al., (2015)**[51] had ameliorated the potential cardioprotective effects of baicalin isoproterenol-induced acute myocardial infarction (AMI) through inducible nitric oxide synthase (iNOS), inflammation, oxidative stress and P38MAPK passageway in rat. Rat model of AMI was induced by isoproterenol (100 mg/kg) and then treated baicalin (various does of baicalin: 1 mg/kg, 10 mg/kg and 100 mg/kg, respectively) for 24 h. Infarct size, the heart weight to body weight ratio and creatine kinase (CK), the MB isoenzyme of creatine kinase (CK-MB), lactate dehydrogenase (LDH) and cardiac troponin T (cTnT) of rats with AMI induced by isoproterenol were used to evaluate curative effect of baicalin on AMI. Meanwhile, iNOS and phosphorylation-p38 MAPK (p-p38) protein expressions, inflammatory factor and oxidative stress were inspected using western blot and commercial kits, respectively. In the present study, pre-treatment with baicalin (10 or 100 mg/kg) significantly ameliorated infarct size, the heart weight to body weight ratio and CK, CK-MB, LDH and cTnT levels in rats with AMI induced by isoproterenol. iNOS protein expression, the serum TNF-α, IL-6, MDA and SOD levels and p-38 protein expressions were significantly suppressed by treatment with baicalin (10 or 100 mg/kg). These results suggest that acute treatment with baicalin ameliorates AMI, iNOS, inflammation, oxidative stress and P38MAPK pathway in rat with AMI induced by isoproterenol.

- **Kumar G et al., (2009)**[52] had studied to assess the effect of *Helicteres isora L.* on four important enzymes of carbohydrate metabolism (glucokinase [GK], hexokinase [HK] phosphofructokinase [PFK] and fructose-1, 6-bisphosphatase [FBP]) along with glycogen content of insulin-dependent (skeletal muscle and liver) and insulin-independent tissues (kidneys and brain) in streptozotocin (STZ; 60 mg/kg)-induced model of diabetes for 30 days. Administration of bark extracts (100, 200 mg/kg) for 30 days led to decrease in plasma glucose levels by approximately 9.60% and 22.04% and 19.18% and 33.93% on 15th and 30th day, respectively, of the experiment. Liver and two-kidney weight expressed as percentage of body weight significantly

increased in diabetics ($P < 0.05$) versus normal controls. Renal glycogen content increased by 10 folds while hepatic and skeletal muscle glycogen content decreased by 75% and 68% in diabetic controls versus controls. *H. isora* did not affect glycogen content in any tissue. The decreased activities of PFK, GK, FBP and HK in diabetic controls were 40%, 50%, 50% and 60% and bark extract of *H. isora* partially corrected this alteration. The efficacy of the bark extract was comparable with Tolbutamide, a well-known hypoglycemic drug.

- **Kumar G et al., (2008)**[53] had evaluated the hypolipidaemic effect of an aqueous extract of the bark of *Helicteres isora* which was investigated in streptozotocin (STZ)-induced diabetic rats. Administration of the bark extract of *Helicteres isora* (100 and 200 mg/kgb.w.) for 21 days resulted in significant reduction in serum and tissue cholesterol, phospholipids, free fatty acids and triglycerides in STZ diabetic rats. In addition to that, significant ($p<0.05$) decrease in high-density lipoprotein (HDL) whereas significant increase ($p<0.05$) low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL) were observed in STZ diabetic rats, which were normalized after 21 days of bark extract treatment. The bark extract at a dose of 200 mg/kgb.w. showed much significant hypolipidaemic effect than at the dose of 100 mg/kgb.w.Wenhao et al. (1991) isolated β-sitosterol, betulic acid, oleanolic acid daucosterolisorin (I) along with 3β,27-diacetoxy-lup-20(29)-en-28-oic methyl ester.

- **Kumar G et al., (2006a)**[54] had investigated the hypoglycaemic effect of the aqueous extract of the bark of *Helicteres isora* L. (Sterculiaceae) in normal, glucose load conditions and streptozotocin (STZ)-induced diabetic rats. In normal rats, the aqueous extract of the bark of *Helicteres isora* L. (100 and 200 mg/kg/p.o.) significantly ($P<0.001$) reduced the blood glucose levels from 64.5-48.5 and 67-47 mg% 2h after oral administration of bark extract and also significantly lowered the blood glucose in STZ diabetic rats from 68-105 and 66-85.5 mg% 21 days after daily oral administration of the extract ($P<0.001$). The results suggested that the aqueous extract of bark of *Helicteres isora L.* possesses a potential hypoglycaemic effect in diabetic rats.

- **Kumar G et al., (2006b)**[55] had examined the effect of oral administration of an aqueous extract of the bark of *Helicteres isora* on blood glucose and plasma antioxidant status in streptozotocin (STZ) induced diabetic rats. The study was also undertaken to evaluate the role of hepatic enzymes in experimental diabetes. Oral administration of a bark extract of *Helicteres isora* (100, 200 mg/kg) in STZ diabetic rats caused a significant increase in body weight, hepatic hexokinase activity and significant decrease in hepatic glucose-6-phosphatase, serum acid phosphatase (ACP), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH). Based on these findings, we suggest that Helicteresisora possesses hypoglycemic and hepatoprotective activity and is able to ameliorate biochemical damage in STZ induced diabetic rats.

# 3. PLANT PROFILE[56, 57, 58]

| | |
|---|---|
| **KINGDOM** | **:**Plantae |
| **CLASS** | **:**Angiosperms |
| **SUBCLASS** | **:**Eudicots |
| **ORDER** | **:**Malvales |
| **FAMILY** | **:**Malvaceae |
| **GENUS** | **:**Helicteres |
| **BOTANICAL NAME** | **:**Helicteresisora(L.) |

## VERNACULAR NAME:

| **Languages** | **-** | **Names** |
|---|---|---|
| Sanskrit | - | Murva, Avartani, Avartaphala |
| Hindi | - | Marodphali, Marorphali, Enthani, Gomathi |
| Marathi | - | Kewad, Muradsheng |
| Bengal | - | Antmora |
| Gujarat | - | Maradashingh, Maradashinghi |
| Tamil | - | Balampari |
| Telugu | - | Guvadarra |
| Kannada | - | Pedamuri |
| Malayalam | - | Ishwarmuri |
| Oriya | - | Murmuriya |
| English | - | East India screw tree, Indian screw tree |

**DISTRIBUTION:**

The plant is found throughout India; from Punjab to Bengal, Jammu to South India. Usually, the shrub or tree grows in dry deciduous forests of Central and Western India up to 1500 m on the hill slopes. It is widely found flora of Central and Western India. It is also found in Malay Peninsula, Java, Australia.

**PLANT:**

It is sub-deciduous small tree or shrub of about 1.5-3.0m height. Young branches are rough with scattered stellate hairs. The leaves are serrate, obliquely cordate or ovate, shortly acuminate and rough above and pubescent beneath. The flowers are solitary or in sparse clusters with red reflexed petals, become pale-blue when old. The fruits are 5.0 cm long, greenish-brown, beaked and cylindrical with 5 spirally twisted carpels. The seeds are tubercled. Fruits, seeds, bark and roots of the plant are used. The flowering time of H. isora is from April to December, and the fruiting time is from October to June.

**PARTS USED:**

Bark (Stem bark)

**CHEMICAL COMPOSITION:**

The bark contains choloplast, pigments, phytosterols, hydroxyl carboxylic acid,orangeyellow colouring matter, saponins, phlobotannis, sugaranglignins.

**PROPERTIES AND USES:**

The bark of *Helicteres isora* Linn. (Sterculiaceae)has been used in indigenous systems of medicine inIndia for the treatment of diabetes mellitus since timeimmemorial.The roots and the bark are expectorantand demulcent and are useful in colic, scabies,gastropathy, diabetes, diarrhoea and dysentery. Theaqueous extract of the bark showed a significanthypoglycaemiceffect,[60]hypolipidaemic activity,[61] lowering effect of hepatic enzymes[62] and glycoprotein levels[59] and an antiperoxidative effect.[63]


**Plant of *Helicteres isora Linn***

**Bark of *Helicteres isora Linn***

# 4. AIM AND OBJECTIVES

The aim of the study is to evaluate the cardioprotective effect of bark of *Helicteres isora* Linn. by carrying out the pharmacological studies with the ethanolic extract of bark of *Helicteres isora* Linn.

The main goal of the study is to develop a new herbal remedy for the Myocardial infarction. Many drugs present in market which is used for the treatment of the disease produce many side effects. Also these drugs mainly cure the symptoms of the disease and not the underlying cause.

The side effects produced by the synthetic agents include dizziness, weakness and increasing the intraocular pressure precipitating glaucoma (nitrates), nausea, vomiting, rashes, wheezing, peripheral edema and constipation caused by Calcium channel antagonists, etc.

On the other hand, herbal drugs produce less side effects and are cost effective to all classes of people. Thus there is a need to replace synthetic agents by safe and effective plant based herbal remedies as cardioprotective agents. Many plants extracts have been used as cardioprotective agent in folklore claim and in traditional medicines.

# 5. PLAN OF WORK

- Collection and authentication of the plant material
- Preparation of extract
- Preliminary phytochemical study of extract
- Pharmacological screening methods
- *INVITRO* METHODS:
    - ECG changes
- *INVIVO* METHODS:
    - Estimation of serum lipid level
    - Estimation of cardiac marker enzymes
    - Antioxidant parameters

# 6. MATERIALS AND METHODS

## COLLECTION AND AUTHENTICATION OF PLANT MATERIALS

The bark of *Helicteres isora* Linn. was collected during December 2015 from Solakkadu, Kollimalai, Namakkal District, Tamilnadu, India, identified and authenticated as *Helicteres isora* Linn. by botanical survey of India, Coimbatore. The voucher specimen was kept in college, (BSI/SRC/2/23/2014-2015/ 1191) for future reference.

## EXTRACTION

The dried bark of *Helicteres isora* Linn. was ground into a fine powder with an auto-mix blender. The fine bark powder was suspended in equal amount of ethanol in soxhlet apparatus. Then the fine powder was suspended in an equal amount of ethanol, stirred intermittently and left overnight. The macerated pulp was then filtered through a coarse sieve and the filtrate was dried at reduced temperature. This dry mass (yield 182.4 g/kg of powdered bark) served as ethanolic extract of *Helicteres isora* Linn. for experimentation. The appearance of resulted extract yield of the extract was dark green color.

## PRELIMINARY PHYTOCHEMICAL ANALYSIS

The ethanolic extract of bark of *Helicteres isora* Linn. was subjected to preliminary phytochemical screening[64, 65, 66]

### 1. Test for Alkaloids

The extracts was treated with diluted Hydrochloric acid and filtered. The filtrate was treated with various alkaloidal agents.

**Mayer's Test**: The extract was treated with Mayer's reagent, appearance of cream colour indicated presence of alkaloids.

**Dragendroff`s Test**: The extract was treated with Dragendroff`s reagent, appearance of reddish brown precipitate indicated presence of alkaloids.

**Hager's Test:**The extract was treated with Hanger's reagent, appearance of yellow colour indicated presence of alkaloids.

**Wager's Test:**The extract was treated with wager's reagent, appearance of brown precipitate was indicated presence of alkaloids

**2. Test for Carbohydrates**

The extracts were treated with 3 ml of alpha naphthol in alcohol and Conc.Sulphuric acid was carefully added to side of the test tubes. Formation of a violet ring at the junction of two liquids indicated the presence of carbohydrates.

**Fehling`s Test:** To the sample Fehling`s solution A and B was added and heated for two minutes. Appearance of reddish brown colour indicated presence of reducing sugars.

**Benedict's Test:** To the sample Benedict's solution was added and heated, appearance of reddish orange precipitate will indicate the presence of reducing sugars.

**Barfoed's Test:** The sample was treated with Barfoed`s reagent and heated, appearance of reddish orange precipitate was indicated presence of reducing sugars.

**3. Test for Proteins**

**Biuret's Test**: To the extracts Copper sulphate solution followed by Sodium hydroxide solution was added, a violet colour precipitates indicated presence of proteins.

**Million's Test:** To the extracts Million's reagent was added, appearance of pink colour indicated presence of proteins.

**4. Test for Steroids**

**LibermannBurchard`s Test:** The extracts was treated with Conc. Sulphuric acid and Glacial acetic acid followed by Acetic anhydride, a violet ring appears at the junction of the liquids and appearance of green colour in the aqueous layer indicated presence of steroids.

**5. Test for Sterols**

The extracts was treated with 5%KOH solution, appearance of pink colour indicated the presence of sterols.

**6. Test for Phenols**

The extracts were treated with neutral Ferric chloride solution, appearance of violet colour indicated presence of phenols.

The extracts were treated with 10% Sodium chloride solution, appearance of cream colour indicated presence of phenols.

**7. Test for Tannins**

The extract was treated with 10% Lead acetate solution appearance of white precipitate indicated presence of tannins.

The extracts were treated with aqueous bromine water; appearance of white precipitate indicated presence of tannins.

**8. Test for Flavanoids**

5ml of the extracts solution was hydrolyzed with 10% Sulphuric acid and cooled. it was then extracted with Diethyl ether and divided in to 3 portions in three separate test tubes.1ml of diluted sodium carbonate, 1 ml of 0.1 N Sodium hydroxide and 1 ml of diluted ammonia solutions was added to the first second and third test tube respectively. Development of yellow colour in each test tube indicated presence of flavanoids.

**Shindoas test**: The extracts were dissolved in alcohol, to which a piece of Magnesium followed by drop wise addition of Conc. Hydrochloric acid and heated. Appearance of magenta colour indicated the presence of flavanoids.

**9. Test for Gums and Mucilage**

The extracts were treated with 25 ml absolute alcohol and then the solution will be filtered. The filtrate was examined for its swelling properties.

**10. Test for Glycosides**

A pinch of the extract was dissolved in Glacial acetic acid and few drops of Ferric chloride solution was added followed by the addition of Conc.Sulphuric acid, formation of red ring at the junction of the two liquids indicated presence of glycosides.

**11. Test for Saponins**

**Foam test**: 1 ml of the extract was diluted to 20 ml with distilled water, formation of foam in the upper part of the test tubes presence of saponins.

**12. Test for Terpenes**

The extracts were treated with tin and Thionyl chloride, appearance of pink colour indicated presence of terpenes.

# PHARMACOLOGICAL STUDIES

## ACUTE TOXICITY STUDIES[67]

Acute toxicity studies were performed by using OECD guide lines (Organization of Economic Cooperation and Development) 423. It's a step wise procedure with 3 female animals per step. Depending upon the mortality and/or morbidity status of animals, the average of 2-3 steps are necessary to allow judgment on the acute toxicity of the test substance. It results in the use of minimum number of animals while allowing for acceptable data based scientific conclusion. The procedure uses defined doses (2000mg/kg body weight) and the result allows a test substance to be ranked and classified according to the Globally Harmonized System (GHS) for the classification of chemicals which cause acute toxicity.

## EXPERIMENTAL PROCEDURE

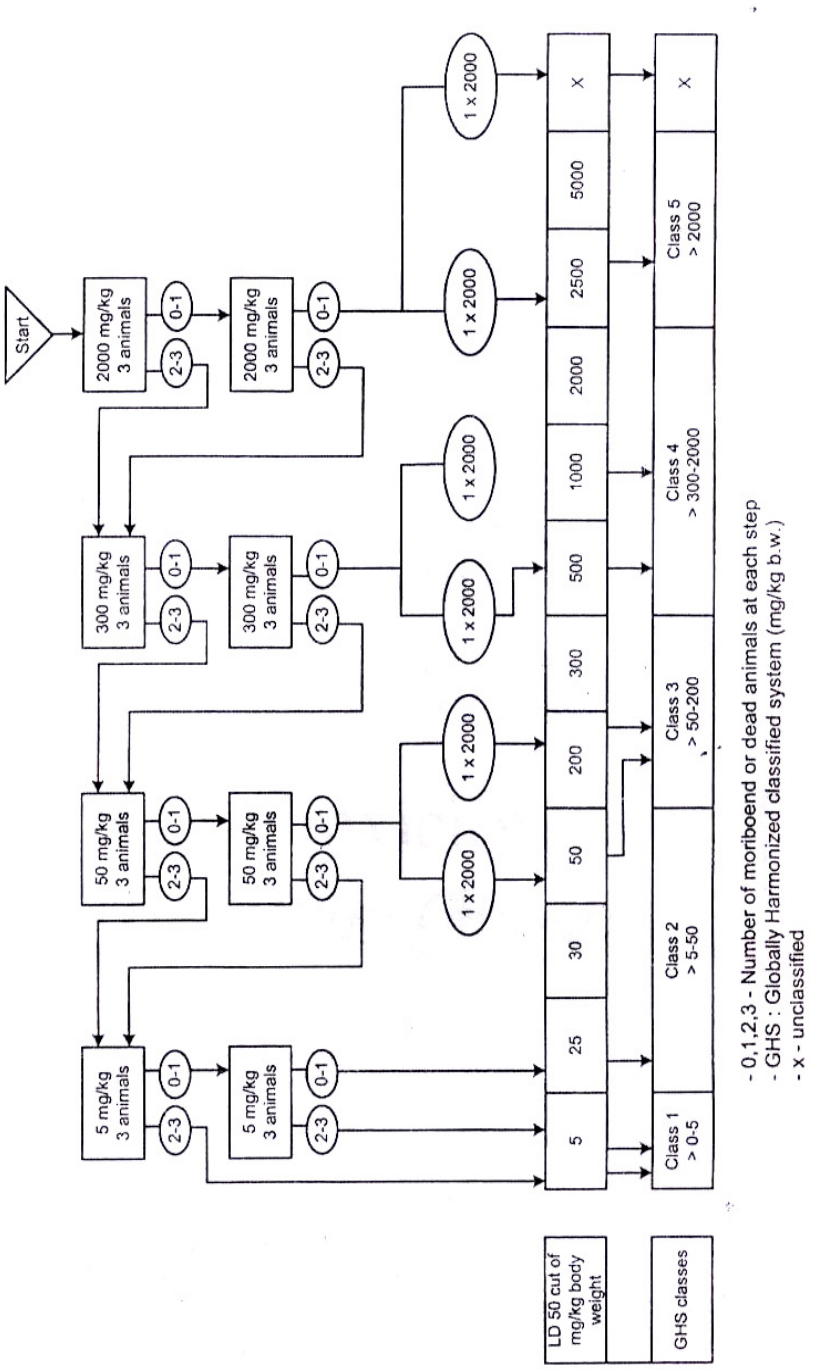
Three male Wistar rats weighing 150-220g body weight were used for the study, since the herbal extract are relatively non-toxic, 2000mg/kg bodyweight was selected as the starting dose level of the extract (defined dose by OECD 423 guidelines). 18 hours prior to the administration of test drug, animals were fasted overnight with water *ad libitum*. Body weight of rats before and after administration of test drug noted and any changes in fur and skin and, eyes and mucous membrane, respiratory system and circulatory system were observed and also sign of convulsion, tremors, diarrheoa, salivation, lethargy, sleep and coma were noted. The onset of toxicity and signs of toxicity were also noted.

**Behavioral profile:** Alertness, irritability, restlessness and fearfulness.

**Neurological profile:** Spontaneous activity, reactivity, touches response and pain response.

**Autonomic profile:** Defecation and urination, lethality or death of animals was observed after 24 hours and 72 hours respectively.

**FIG : OECD Guidelines for Acute Oral Toxicity**



## EXPERIMENTAL ANIMALS

Colony inbreed strains of Male Wistar rats of 160-200g body weight were used for pharmacological studies, three different groups with 6 animals in each, were housed at controlled temperature (25±2°C), humidity (60–80% relative humidity) and light dark cycle (12/12 hour); on a standard rodent chow (Hindustan lever pvt ltd., Bangalore) and water *ad libitum*. Animals were handled carefully and acclimatized to laboratory conditions for 14 days prior to experimentation. All the experimental procedures and protocols used in this study were reviewed by the Institutional Animal Ethical Committee (887/2PO/Re/S/2005/CPSCEA), and studies were carried out in accordance with Institutional ethical guidelines.

The animals were fasted and no water, from 12 hours prior to the application of anesthesia, which preceded the sacrifice, blood collection, whose aim was to perform complete blood count and fragments of the left ventricle.

## INDUCTION OF MYOCARDIAL INFARCTION:[68]

After the treatment of animals for three weeks with aqueous extract of bark of *Helicteres isora* Linn., isoproterenol (ISO) 85 mg/kg/day (diluted in 2 ml of saline), *s.c* was administered below the skin of the neck in two divided doses at 12 hours intervals to induce myocardial necrosis. Twenty four hour after the first dose of ISO administration, the rats were sacrificed.

## METHODOLOGY

The animals were divided into three groups of 6 animals each. Group-1 received normal saline and was considered as normal control animals. Group-2 received normal saline. Group-3 received ethanolic extract of bark of *Helicteres isora* Linn. in a dose of 400 mg/kg/p.o. The treatment was continued for about 3 weeks. After completion of treatment, all groups of animals except for group 1were fasted overnight and were induced Myocardial Infarction (MI) by giving subcutaneous injection of 85mg/kg of ISO in two divided doses at 12 hours interval. Twenty Four hour after the first dose of ISO administration, ECG of rats taken and were sacrificed.

## ANESTHESIA

Twenty-four hours after the last subcutaneous injection, anesthesia was proceeded to perform the sacrifice of animals. The anesthetic technique involved the use of thiopentone Sodium anaesthesia at the dose of 30 mg/kg applied intraperitoneally.

## ECG CHANGES

Needle electrodes were placed subcutaneously and changes in Lead II were recorded 12hours after the second dose of ISO on an electrocardiograph. Heart rate and electrocardiograph [ECG] were observed in anesthetized animals (Thiopentone Sodium anaesthesia 30 mg/kg i.p.)for a period of one minute for every 5 minutes. The type of alterations [ST-segment elevation or depression] in normal and experimental animals was considered.

## COLLECTION OF SAMPLES

After adequate anesthesia, an incision was performed in an inverted T extending from neck to pubis.

## COLLECTION OF BLOOD

We obtained access to the retroperitoneum, the abdominal aorta was dissected in its infrarenal portion. The blood collection was then performed through a puncture of the abdominal aorta with a disposable Jelco number 24 (24 gauge). Using a 5 mL disposable syringe, coupled with Jelco 5 mL of blood were collected. Of this volume, 2 mL were stored in a tube for complete blood count and 3 mL in a tube for biochemical dosages (urea, creatinine, SGOT, SGPT and troponin I). The tubes, properly identified, were then placed in a cooler with ice.

## COLLECTION OF PLASMA SERUM

Blood was collected from the retroperitoneum, and serum was separated by centrifuging the blood at 3000 rpm after the blood clots. The supernatant serum was separated and used for estimation of marker enzymes.

## COLLECTION OF FRAGMENTS OF THE MYOCARDIUM TO MEASURE THE ACTIVITY OF THE ENZYME LEVELS

After the withdrawal phase of the cardiac apex, we proceeded immediately to the resection of two fragments of the left ventricle muscle. These fragments were weighed on a precision balance, and placed separately inside cryotubes properly identified and put into container of liquid nitrogen. The cryovials were then stored in a freezer at a temperature of -70ºC until the analysis in a period of up to two weeks.

## ESTIMATION OF SERUM LIPID PROFILE

The levels of HDL, LDL and VLDL were estimated in the supernatant serum obtained by using commercially available kits (HIMEDIA, Bangalore).

## ESTIMATION OF CARDIAC MARKER ENZYMES

### ➢ ESTIMATION OF CREATININE-KINASE

Blood serum was isolated and 10% homogenate was prepared by adding 50mM Phosphate buffer, pH 7.4. and homogenate was centrifuged at 7000 rpm for 15 mins. and the supernatant used for the estimation of CK-MB by following the procedure given in the kits.

### ➢ ESTIMATION OF LACTATE DEHYDROGENASE (LDH)[69]

Blood serum was homogenised in 5ml of 0.1 M Tris-Hydrochloride buffer in ice cold condition. The homogenate was centrifuged at 2500 rpm for 5 mins. The supernatant was used for the estimation of LDH following the procedure given in the kits.

## ANTIOXIDANT PARAMETERS

### ▪ ESTIMATION OF LIPID PEROXIDATION[70]

**PROCEDURE:**

Lipid peroxidation products of heart homogenate were determined as thiobarbituric acid reactive substances (TBARS). Heart of the Control and treated rats was homogenised in ice-cold 0.9% saline to get 10% homogenate. 0.5 ml of supernatant after differential

centrifugation was allowed to react with 3 ml of 1% Orthophosphoric acid and 1 ml of 0.6% thiobarbituric acid. The tubes was heated in boiling waterbath for 45 mins, cooled and then 4 ml of n-butanol was added to each test tube mixed vigorously and centrifuged for 5 mins at 1000 rpm. The supernatant was used for determination of TBARS at 535 nm against a reagent blank. TBARS were expressed as nmol/L wet weight.

- **ESTIMATION OF SUPEROXIDE DISMUTASE (SOD)**[71]

**PROCEDURE:**

0.1 ml of homogenate was taken in a test tube containing 1.2 ml of sodium pyrophosphate buffer (pH 8.3,0.052M). To this mixture 0.1ml of Phenazinemethosulphate (186 µM) and 0.3ml of 300µM Nitrobluetetrazolium will added. The reaction was started by addition of 0.2 ml of NADH (750µM) the reaction mixture was incubated for 90 sec at 30°C. The reaction was stopped by addition of 0.1ml glacial acetic acid. Then the mixture was stirred vigorously with 4.0 ml of n-butanol and allowed to stand for 10mins to separate butanol layer. The butanol layer was separated and used for determination of SOD at 560 nm. The activity of SOD was expressed as units/mg protein.

- **ESTIMATION OF REDUCED GLUTATHIONE (GSH)**[72]

**PROCEDURE:**

0.5 ml of homogenate was added to each tube containing 0.5 ml Trichloroacetic acid (TCA 10%). The test tube was gently shaken intermittently for 10 mins followed by centrifugation at 3000 rpm for 5mins at room temperature. Accurately 0.1 ml of the resulting clear supernatant was mixed with 1.8ml of the phosphate buffer (0.1M, pH 8) in separate test tubes. Atleast a duplicate was made for each sample. 0.1 ml Ellman's reagent (0.39%) was added to each tube and after 5mins, the optical density was measured at 412 nm against a reagent blank. The data was expressed as mmol/g tissue.

## STATISTICAL ANALYSIS

The statistical analysis was carried by one way ANOVA followed by Dunnet's "t" test. (P values < 0.001) was considered statistically significant, it was calculated using graph pad prism Version 6.

# 7. RESULTS

**PREPARATION OF PLANT EXTRACTS:**

In Soxhlet apparatus, the dried 1000 g of *Helicteres isora* Linn. bark powder was taken for extraction for 24 hours. Amount of the extract of *Helicteres isora* Linn. obtained was 182.4 g. Percentage yield was found to be 18.24%.

**PRELIMINARY PHYTOCHEMICAL ANALYSIS OF THE EXTRACT OF *Helicteres isora* Linn. BARK:**

The results of preliminary phytochemical analysis of the ethanolic extract of bark of *Helicteres isora* Linn. is shown in **Table 1.**The ethanolic extract of bark of *Helicteres isora* Linn. showed the presence of various phytochemical constituents such as

**TABLE 1: List of Phytochemical Constituents Screened in *Helicteres isora* Linn.**

| S.No. | Constituents | Ethanolic Extract |
|---|---|---|
| 1. | Alkaloids | + |
| 2. | Carbohydrates | + |
| 3. | Protein | _ |
| 4. | Steroids | + |
| 5. | Phenols | + |
| 6. | Tannins | _ |
| 7. | Flavonoids | + |
| 8. | Gums and Mucilage | _ |
| 9. | Glycosides | + |
| 10. | Saponins | + |
| 11. | Terpenoids | + |
| 12. | Cardiac Glycosides | _ |

**+** Present;**–** Absent;

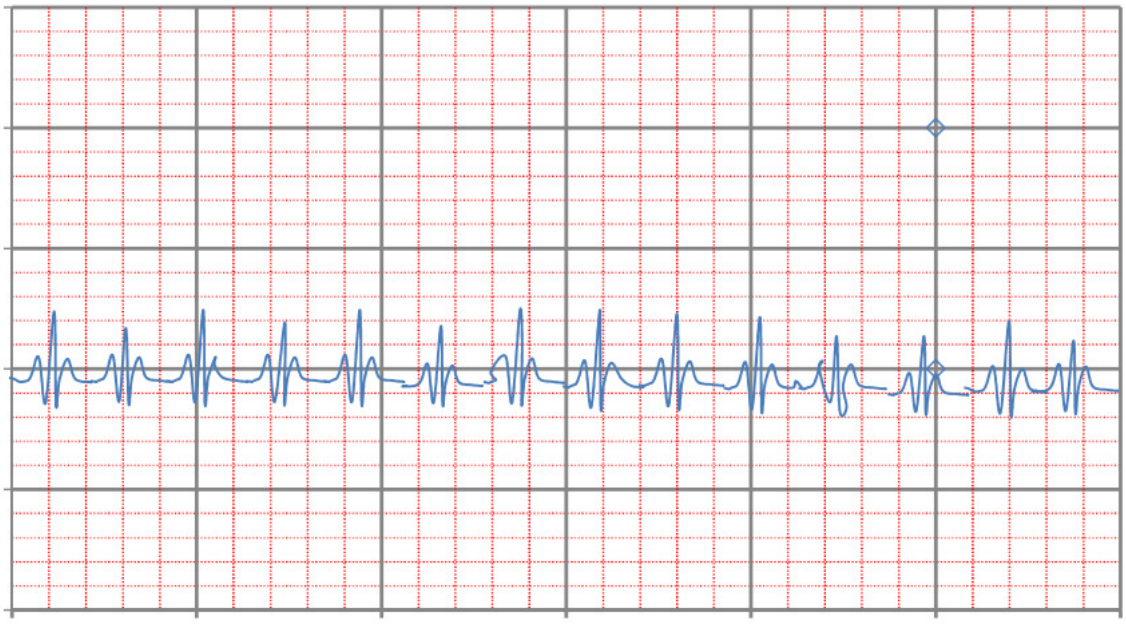
## ACUTE ORAL TOXICITY STUDY

The acute oral toxicity study was performed according to OECD 423 guidelines. A single oral administration of a starting dose of 2000 mg/kg body weight, of ethanolic extract of bark of *Helicteres isora* Linn. (EBHI) was administered to 3 male rats and observed. There was no lethality, mortality or any toxic reactions found at any selected dose level until the end of the study period. The results of acute oral toxicity studies are shown in **Table 2.**

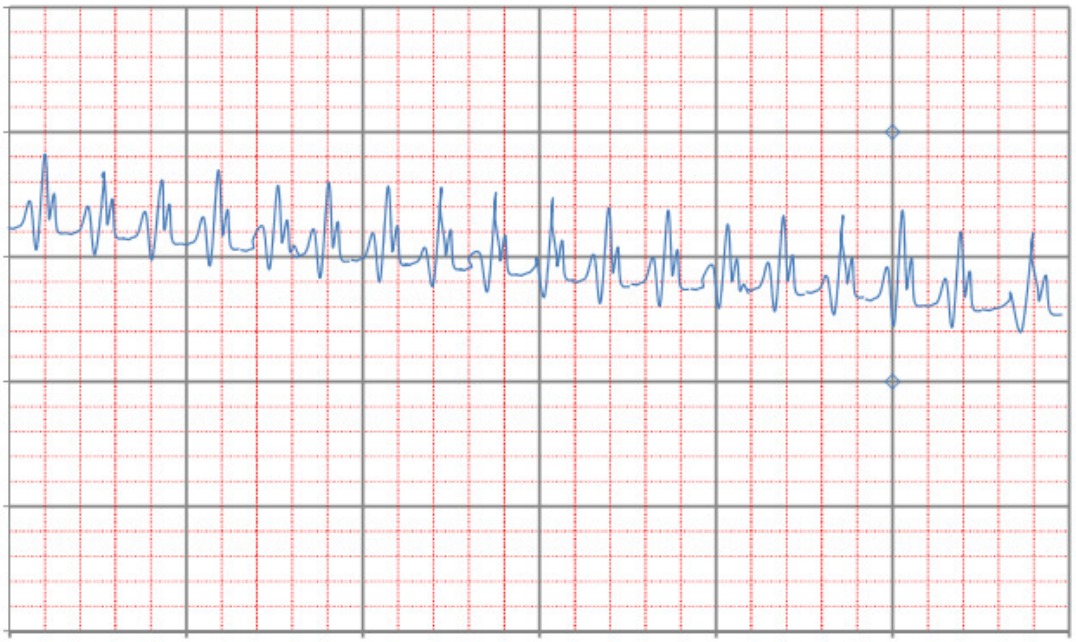
**TABLE 2: Acute Toxicity Study of *Helicteres isora* Linn.**

| S. No. | Treatment group | Dose | Weight of animal in gms | | Signs of toxicity | Onset of toxicity | Reversible or irreversible | Duration |
|---|---|---|---|---|---|---|---|---|
| | | | Before Test | After test | | | | |
| 1. | EBHI | 2g/kg | 150 | 155 | No signs of toxicity | Nil | Nil | 14 days |
| 2. | EBHI | 2g/kg | 150 | 170 | No signs of toxicity | Nil | Nil | 14 days |
| 3. | EBHI | 2g/kg | 170 | 185 | No signs of toxicity | Nil | Nil | 14 days |

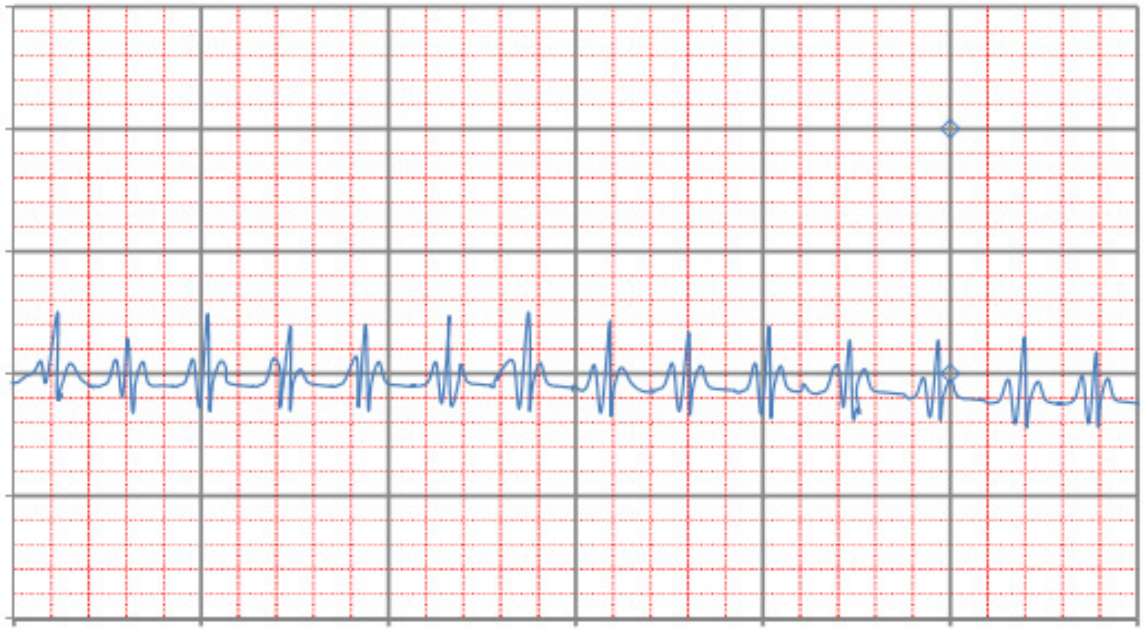
## ECG CHANGES



**Group 1 (control)**



**Group 2 ( Negative control)**



**Group 3 (EBHI )**

**Ck-MB LEVELS**

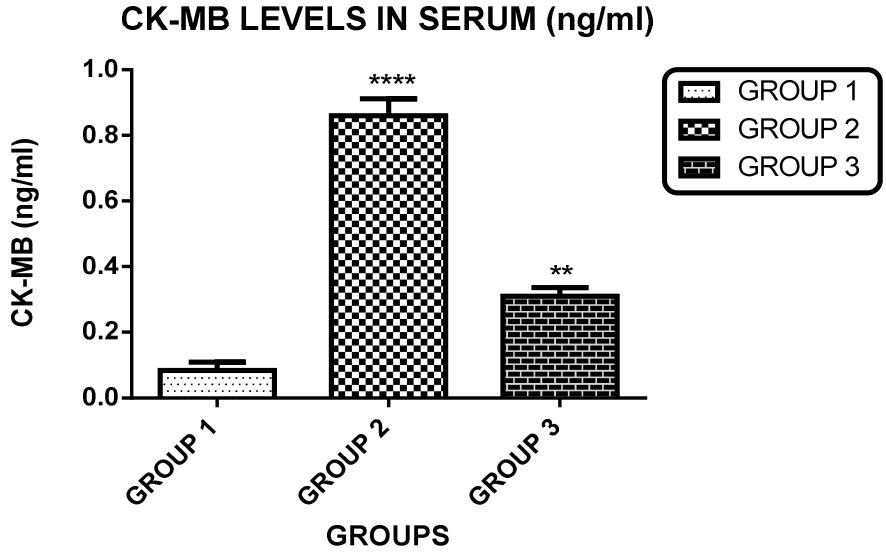
Table 3 indicates that the change which occurs due to the pretrearment of ethanolic extract of bark of *Helicteres isora* Linn. in CK-MB enzyme level in the blood serum.

**TABLE 3: Effect of Ethanolic extract of *Helicteres isora* on CK-MB levels in serum**

| GROUPS | CK-MB (ng/ml) |
|---|---|
| **GROUP 1 (CONTROL)** | 0.08±0.026 |
| **GROUP 2 (NEGATIVE CONTROL)** | 0.86±0.052**** |
| **GROUP 3 (400 mg/kg EBHI)** | 0.31±0.026** |

Values (ng/ml) are expressed as mean ± SEM (n=6) .Values comparison were made between Group I Vs Group II, III (**** p <0.0001, ** p<0.01).

**FIG.9: Effect of Ethanolic extract of *Helicteres isora* on CK-MB levels in serum**



## LACTATE DEHYDROGENASE LEVELS

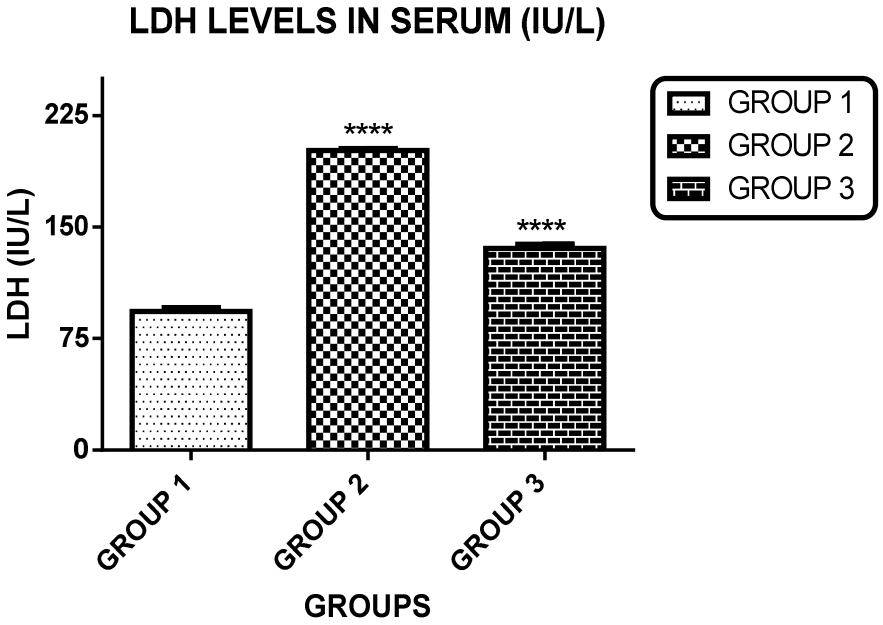
**Table 4** indicates the changes occur due to the pretreatment of *Helicteres isora* Linn. in the (Lactate Dehydrogenase) LDH enzyme level in the blood serum.

**TABLE 4: Effect of Ethanolic extract of *Helicteres isora* on LDH levels in serum**

| GROUPS | LDH (IU/L) |
|---|---|
| **GROUP 1 (CONTROL)** | 93.15 ± 2.73 |
| **GROUP 2 (NEGATIVE CONTROL)** | 201.60 ± 1.18**** |
| **GROUP 3 (400 mg/kg EBHI)** | 135.70 ± 2.71**** |

Values (IU/l) are expressed as mean ± SEM (n=6) .Values comparison were made between Group I Vs Group II, III (****p <0.0001).

**FIG. 10: Effect of Ethanolic extract of *Helicteres isora* on LDH levels in serum**



## LIPID PROFILE

For the estimation of lipid profile, blood is withdrawn by retroperitoneal puncture and LDL, HDL, Total cholesterol levels are estimated.

## LOW DENSITY LIPOPROTEIN LEVELS

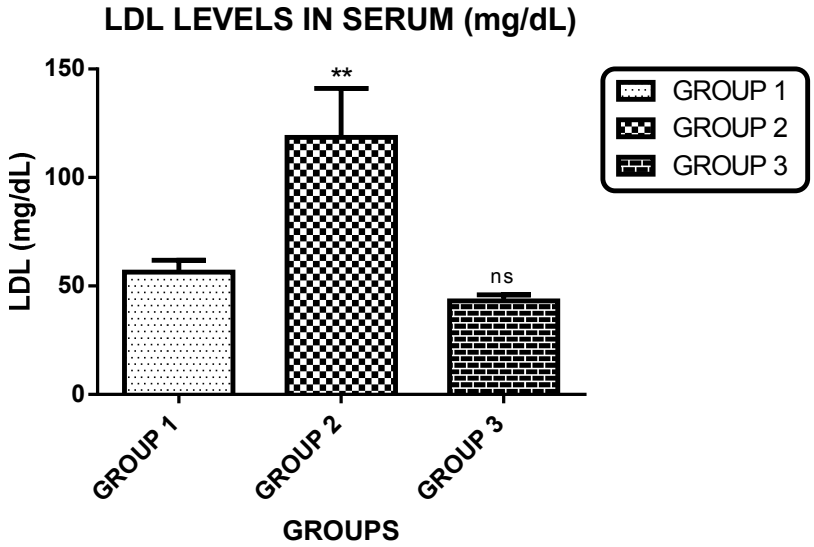
Low Density Lipoprotein(LDL) levels were estimated in the serum of experimental animals and the values obtained are shown in **table 5**.

**TABLE 5: Effect of Ethanolic extract of *Helicteres isora* on LDL levels in serum**

| GROUPS | LDL (mg $dL^{-1}$) |
|---|---|
| **GROUP 1 (CONTROL)** | 56.30 ± 5.476 |
| **GROUP 2 (NEGATIVE CONTROL)** | 118.50 ± 22.560** |
| **GROUP 3 (400 mg/kg EBHI)** | 43.02 ± 2.934ns |

Values (**mg $dL^{-1}$**) are expressed as mean ± SEM (n=6) .Values comparison were made between Group I Vs GroupII, III (**p <0.01, ns – not significant).

**FIG.11: Effect of Ethanolic extract of *Helicteres isora* on LDL levels in serum**



## HIGH DENSITY LIPOPROTEIN LEVELS

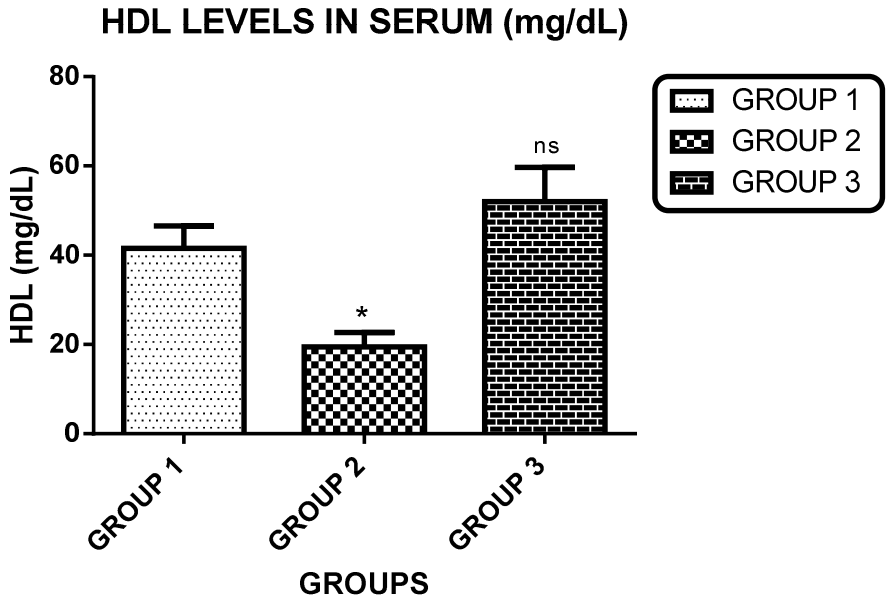
High Density Lipoprotein (HDL) levels were estimated in the serum of the experimental animals and the values obtained are shown in **table 6**.

**TABLE 6: Effect of Ethanolic extract of *Helicteres isora* on HDL levels in serum**

| GROUPS | HDL (mg $dL^{-1}$) |
|---|---|
| **GROUP 1 (CONTROL)** | 41.59 ± 4.946 |
| **GROUP 2 (NEGATIVE CONTROL)** | 19.46 ± 3.232* |
| **GROUP 3 (400 mg/kg EBHI)** | 52.07 ± 7.639ns |

Values (**mg $dL^{-1}$**) are expressed as mean ± SEM (n=6) .Values comparison were made between Group I Vs Group II, III (**p <0.01, ns – not significant).

**FIG. 12: Effect of Ethanolic extract of *Helicteres isora* on HDL levels in serum**



## TOTAL CHOLESTEROL LEVELS

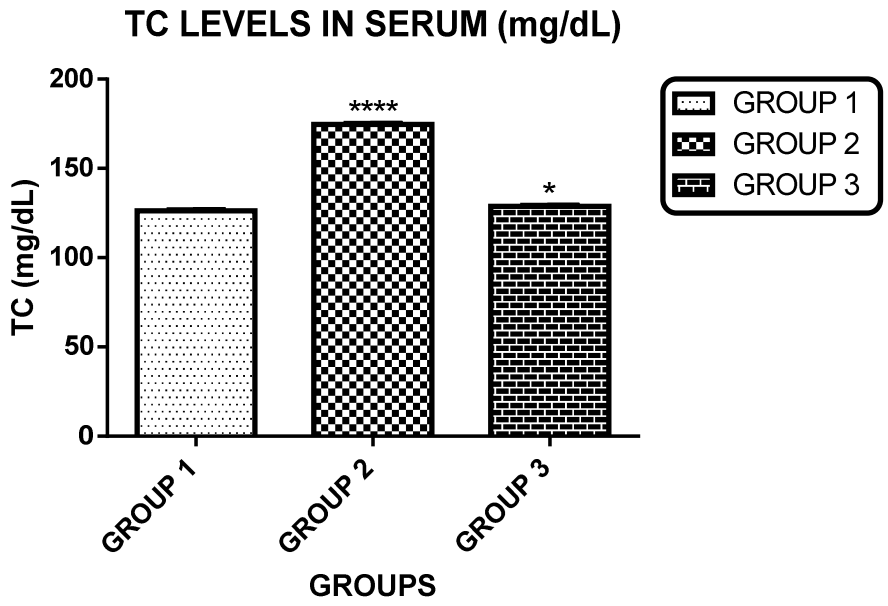
Total Cholesterol levels were estimated in the serum of the experimental animals and the values obtained are shown in **table 7**.

**TABLE 7: Effect of Ethanolic extract of *Helicteres isora* on Total Cholesterol levels in serum**

| GROUPS | TOTAL CHOLESTEROL (mg $dL^{-1}$) |
|---|---|
| **GROUP 1 (CONTROL)** | 126.2 ± 0.756 |
| **GROUP 2 (NEGATIVE CONTROL)** | 174.7 ± 0.647**** |
| **GROUP 3 (400 mg/kg EBHI)** | 128.8 ± 0.696* |

Values (**mg $dL^{-1}$**) are expressed as mean ± SEM (n=6) .Value comparison were made between Group I Vs Group II, III (**** $p<0.0001$, * $p<0.1$).

**FIG 13: Effect of Ethanolic extract of *Helicteres isora* on Total Cholesterol levels in serum**



## ANTIOXIDANTS

## LIPID PEROXIDATION

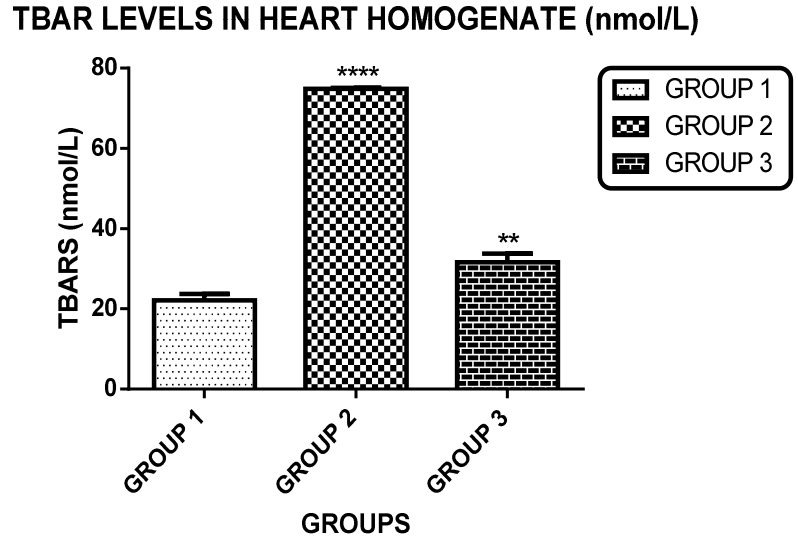
**Table 8** indicates that the TBARS levels in the heart tissue, which was measured by making heart homogenate. The study indicated that due to Pretreatment of EBHI, decreases in the TBARS levels were found.

**TABLE 8: Effect of Ethanolic extract of *Helicteres isora* on TBARS level in heart homogenate**

| GROUPS | TBARS (nmol/lt) |
|---|---|
| **GROUP 1 (CONTROL)** | 22.07 ± 1.711 |
| **GROUP 2 (NEGATIVE CONTROL)** | 74.81 ± 0.288$^{****}$ |
| **GROUP 3 (400 mg/kg EBHI)** | 31.65 ± 2.135$^{**}$ |

Values were expressed as mean ± SEM (n=6) .Values comparison were made between Group I Vs Group II, III ($^{****}$p <0.0001, $^{**}$p<0.01).

**FIG 14: Effect of Ethanolic extract of *Helicteres isora* on TBARS level in heart homogenate**



**REDUCED GLUTATHIONE**

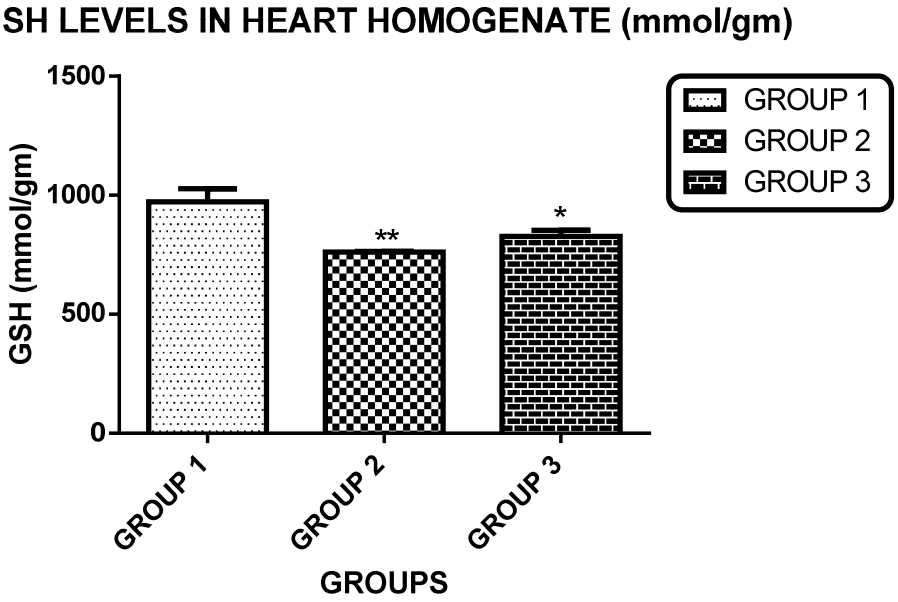
**Table 9** indicates that the Reduced Glutathione (GSH) levels in the heart tissues, which were measured by making heart homogenate. The study indicated that due to Pretreatment of EBHI, increase in the GSH levels was found.

**TABLE 9: Effect of Ethanolic extract of *Helicteres isora* on GSH levels in heart homogenate**

| GROUPS | GSH (mmol/gm) |
|---|---|
| **GROUP 1 (CONTROL)** | 971.5 ± 55.440 |
| **GROUP 2 (NEGATIVE CONTROL)** | 760.8 ± 2.893** |
| **GROUP 3 (400 mg/kg EBHI)** | 826.4 ± 25.950* |

Values are expressed as mean ± SEM (n=6) .Values comparison were made between Group I Vs Group II, III (**p <0.01, *p<0.05).

**FIG 15: Effect of Ethanolic extract of *Helicteres isora* on GSH levels in heart homogenate**



**SUPEROXIDE DISMUTASE**

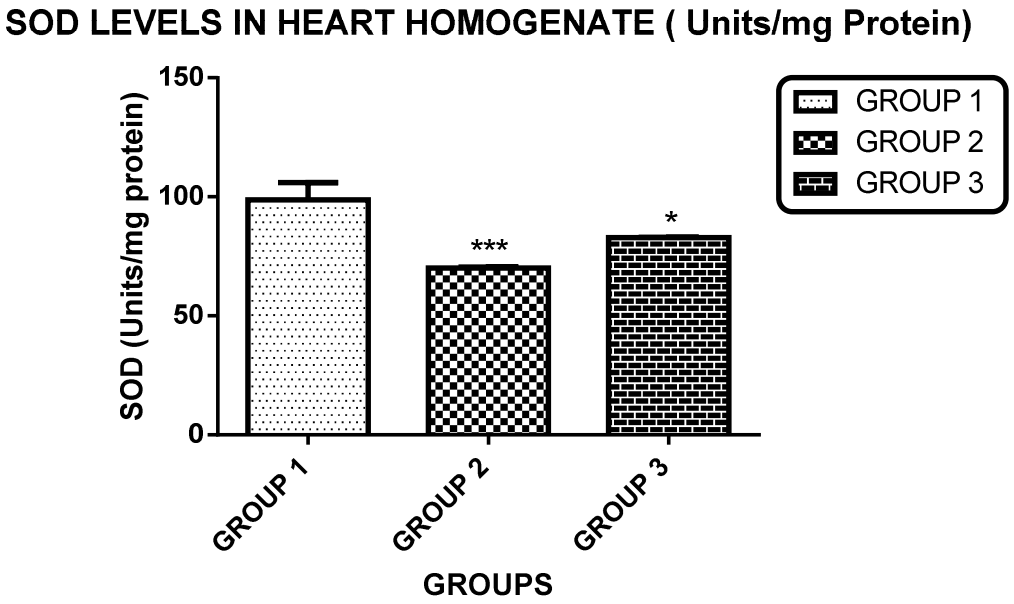
**Table 10** indicates that the Superoxide Dismutase (SOD) levels in the heart tissues, which were measured by making heart homogenate. The study indicated that due to Pretreatment of EBHI, increase in the SOD levels was found.

**TABLE 10: Effect of Ethanolic extract of *Helicteres isora* on SOD levels in heart homogenate**

| GROUPS | SOD (Units/mg protein) |
|---|---|
| **GROUP 1 (CONTROL)** | 98.65 ± 7.324 |
| **GROUP 2 (NEGATIVE CONTROL)** | 70.01 ± 0.577*** |
| **GROUP 3 (400 mg/kg EBHI)** | 82.79 ± 0.259* |

Values are expressed as mean ± SEM (n=6) .Values comparison were made between Group I Vs Group II, III (*** p <0.001, * p<0.05).

**FIG 16: Effect of Ethanolic extract of *Helicteres isora* on SOD levels in heart homogenate**



# 8. DISCUSSION

From long time, plants have been used as source of drugs for the treatment of various ailments in developed as well as developing countries. In recent years, more than 100 medicinal plants are mentioned in the Indian system of medicine including folk medicines for the management of MI, which are effective either separately or in combination. In the present study, the ethanolic extract of bark of *Helicteres isora* Linn. was evaluated for its cardioprotective and antioxidant studies in experimental rats.

Preliminary phytochemical analysis of the ethanolic extract of bark of *Helicteres isora* Linn. showed the presence of phytochemical such as Alkaloids, Carbohydrate, Steroids, Phenols, Flavanoids, Saponins, Terpenoids, Glycosides.

Acute oral toxicity studies of ethanolic extract of bark of *Helicteres isora* Linn. were performed by using OECD 423 guidelines. Studies did not exhibit any lethality or any profound toxic reactions at a dose of 2000 mg/kg/p.o. According to OECD 423 guidelines for acute oral toxicity study, $LD_{50}$ dose of 2000 mg/kg/p.o of ethanolic extract of bark of *Helicteres isora* Linn. was found to be safe.

The formation of plaques in coronary artery decreases the blood flow to the myocardium and leads to hypoxia ultimately resulting in the development of MI. Isoproterenol, a synthetic catecholamine and β-adrenergic agonist, acts through β-adrenergic receptor which causes severe stress in the Myocardium.[73] ISO is the Catecholamine which produces highly toxic free radicals through auto oxidation mechanism, the free radicals produced by this mechanism is highly toxic than other methods of induction.[74] ISO is proposed as a cardiotoxic agent due to its ability destruct myocardial cells, causing hypoxia, and it disturbs the coronary microcirculation and it elevates the intracellular calcium load. Two consecutive doses of ISO by subcutaneous route leads to myocardial cell death.

The cardioprotective activity of ethanolic extract of bark of *Helicteres isora* Linn. (400 mg/kg) is observed by the estimation of the two cardiac marker enzymes CK-MB, LDH in heart. Due to leakage from the heart and into the blood, after induction of MI there is an increase in the cardiac marker enzyme levels in the serum. The protective activity of ethanolic extract of bark of *Helicteres isora* Linn. (400 mg/kg) produces significant actions against ISO induced MI. The significant rise observed in the levels of cardiac marker enzyme

in group-II compared to group-I indicates the severity of the necrotic damage to the myocardial membrane. Enzymes are the best markers of tissue damage because of their specificity and catalytic activity to the tissue. The release of cellular enzymes creates non specific alterations in the membrane integrity and permeability as a response to β-adrenergic stimulation.[75]

The ECG recordings are done to identify changes occurring in the myocardium during MI. ECG abnormalities are important tools for accurate diagnosis of Myocardial infarction. ST segment elevation was observed in ISO induced MI in rat. The study shows significant alterations of ECG graph which were observed in ISO administered rats as compared to normal control rats. Due to ST segment elevation the intensity of P-wave is reduced along with decrease in QRS complex, R-R intervals, QT interval and prolongation of cardiac cycle and insignificant increase in heart rate. The alterations could be due to the consecutive loss of cell membrane in injured myocardium.[76]

In this study, an elevation of ST-segments was observed in ISO induced rat and pretreatment with ethanolic extract of bark of *Helicteres isora* Linn. (400 mg/kg) significantly inhibited ISO induced ST segment elevation suggestive of its cell membrane protecting effects.[77] The appearance of Q-wave and ST segment elevation are some of the indicative signs of ischemia. In the present study no pathological Q wave was observed due to conditions of ischemia. Administration of ethanolic extract of bark of *Helicteres isora* Linn. (400 mg/kg) eliminates the acute fatal complications by protecting the cell membrane damage.

In the present study animals were treated with a dose of ethanolic extract of bark of *Helicteres isora* Linn. (400 mg/kg), and negative control group is treated with ISO (85 mg/kg). The blood serum was collected and estimated for the levels of LDL, HDL, and Total cholesterol. While compared to control group, LDL, Total cholesterol levels were increased in negative control group. HDL level was decreased in negative control when compared to the normal animals. In the extract treated groups LDL, Total cholesterol levels are decreased and HDL levels increased.

A significant increase in the levels of lipid peroxides in heart homogenate on administration of ISO indicates the elevation of lipid peroxidation by free radicals. Due to increase in lipid peroxidation, GSH and SOD levels were lowered significantly in tissue of

negative control group. Glutathione participates directly in destruction of hydrogen peroxide and also promotes the increase in GSH, SOD levels. The pretreatment with ethanolic extract of bark of *Helicteres isora* Linn. (400 mg/kg) decreased the lipid peroxidation level and maintained GSH and SOD levels in tissue.[78]

It is concluded that the ethanolic extract of bark of *Helicteres isora* Linn. (400 mg/kg) extract actively inhibit the Cardiotoxic effects produced by ISO and posses a significant therapeutic value in the prophylactic treatment of Myocardial infarction.

The result obtained from the study indicates that, ethanolic extract of bark of *Helicteres isora* Linn. (400 mg/kg) pretreatment possess significant protection to myocardium against Isoproterenol induced Myocardial infarction.

# 9. CONCLUSION

From our study it may be concluded that the ethanolic extract of bark of *Helicteres isora* Linn. contains phytochemicals such as Alkaloids, Carbohydrates, Steroids, Phenols, Flavonoids, Glycosides, Saponins, Terpenoids.

Acute oral toxicity studies of ethanolic extract of bark of *Helicteres isora* Linn .did not produce any mortality or signs of toxicity at the dose of 2000 mg/kg b.w/p.o, in experimental rats.

In this study, an elevation of ST segments were observed in ISO induced rat and pretreatment with ethanolic extract of bark of *Helicteres isora* Linn. (400 mg/kg) significantly inhibited ISO induced ST segment elevation suggestive of its cell membrane protecting effects.

The ethanolic extract of bark of *Helicteres isora* Linn. (400 mg/kg) possess good cardioprotective activity against Isoproterenol induced myocardial necrosis by decreasing the level of CK-MB, LDH in serum and increase in heart.

On treating with ethanolic extract of bark of *Helicteres isora* Linn. (400 mg/kg), LDL, Total cholesterol levels are decreased and HDL levels increased, while compared to negative control group where HDL level was decreased, and LDL and total cholesterol levels were increased.

GSH & SOD levels were also increased in ethanolic extract of bark of *Helicteres isora* Linn. treated group. The ethanolic extract of bark of *Helicteres isora* Linn. was found to be most effective in the functional recovery of the heart.

Further isolation, characterization and purification of the active constituents and further experimentation would be necessary to elucidate the exact mechanism of action of *Helicteres isora* Linn.

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